Taxonomy and Systematics of the Bangiales (Rhodophyta) in South Africa using an Integrative Approach

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ABSTRACT

The Bangiales is a globally distributed red algal order that is best known for its economic value in the nori industry. The morphological simplicity of the Bangiales offers limited distinguishing characters for taxonomy and the order was therefore broadly classified into two genera based on morphology: the bladed *Porphyra* and filamentous *Bangia*. However, in 2011, a taxonomic revision of the Bangiales based on a two-gene phylogeny identified 15 genera. Since then, an additional bladed genus and numerous species have been added to the order.

The Bangiales were first recorded in South Africa in 1843 when *Porphyra capensis* Kützing was described. Since then several changes have been made to the bangialean flora of South Africa with many new species discovered based on morphological identification. In 2004, a preliminary molecular assessment of *Poprhyra* along the South African coast revealed far greater species diversity than previously recorded. Following the taxonomic revision by Sutherland *et al*. (2011), some species from South Africa have been transferred to the genus *Pyropia*, others remain in *Porphyra* and many others have not yet been re-assessed. At present, three genera; the filamentous, *Bangia* and the bladed *Porphyra* and *Pyropia* have been recorded in South Africa, and comprise numerous species (based on morphology) and molecular entities.

In the present study a comprehensive collection of newly collected and herbarium specimens (collectively *ca*. 300 specimens from *ca*. 50 sites) of the Bangiales from South Africa was analysed. A total of 241 sequences were generated for three unlinked loci (nSSU, *rbc*L & *cox*1). Taxa were identified or delimited via an integrative taxonomic approach using molecular, morpho-anatomical and ecological data. Species were delimited using three DNA-based species delimitation methods (ABGD, GMYC, PTP) applied to the mitochondrial gene *cox*1 (*n*=203) and the plastid gene *rbc*L (*n*=80). A multigene phylogeny was also constructed (nSSU, *rbc*L & *cox*1) and used to delimit species. Subsequent morpho-anatomical analyses complemented with ecological data and herbarium specimens (South Africa and Namibia) showed that 16 species in three genera (11 *Porphyra*, four *Pyropia* and one *Bangia*) are present along the South African coast. Morpho-anatomical characters of two species with uncertain taxonomic status were consistent with the descriptions of two widespread species, *Bangia* cf. *fuscopurpurea* and *Py*. cf. *suborbiculata* but remain to be confirmed using a molecular approach. In addition, two new species, *Pyropia meridionalis* sp. nov. and *Porphyra agulhensis* sp. nov. were described.

Pyropia meridionalis is a kelp-associated species that is commonly found on the kelp limpet, *Cymbula compressa*, or on the stipes of *Ecklonia maxima*, and rarely on other species of southern African kelp, *Laminaria pallida* and *E. radiata* or other algae. This species occurs along the south-west and west coast of South Africa throughout the year, but may extend to Namibia.

Pyropia meridionalis was shown to be previously misidentified as *Py. gardneri* in South Africa. This species was not closely related to other southern African endemic species of *Pyropia*, suggesting that species colonized and spread along this coastline independently. Nevertheless, most species shared close genetic affinities to other Southern Hemisphere taxa. This supports the notion of historic connectivity in the Southern Ocean proposed for red algae.

Porphyra agulhensis is characterized by delicate laciniate rosette blades and a distinct greenish to pale pinkish-purple colour. This species was shown to be historically misidentified as *P. capensis* and is restricted to the Agulhas Marine Province on the south coast of South Africa. It includes one cryptic species (RSAj). The remaining eight molecular species of *Porphyra* formed a monophyletic group and occurred along the Benguela Marine Province on the west coast of South Africa. No single morpho-anatomical or ecological character could distinguish between these molecular species. Despite overlapping conventional morpho-anatomical or ecological characters among cryptic species, all features were within the range of the current description of *P. capensis* and were therefore referred to as the *P. capensis* cryptic species complex (PCC). High genetic diversity and several major lineages were identified in the PCC along the Benguela Marine Province. Conversely, the *Porphyra agulhensis* cryptic species duo along the Agulhas Marine Province presented low levels of genetic variation with *ca*. 70% of individuals belonging to a single haplotype group. Genetic diversity within *Porphyra* in South Africa was higher on the west coast of South Africa than on the south coast and the region between Cape Agulhas and Cape Point was identified as a region of major biogeographic change. Historic and contemporary processes, which likely shape present-day genetic patterns in South African *Porphyra*, are discussed. Similar to *Pyropia*, species of *Porphyra* from South Africa shared a close phylogenetic affinity with some Chilean bladed Bangiales, providing further support for historic connectivity in these red algae in the Southern Ocean.

South Africa is now home to the second highest number of species of *Porphyra* in the world and shares three species of *Pyropia* with Namibia (based on morphological identification). All species identified using molecular sequences appear to be endemic to South Africa or southern Africa. The extensive genetic diversity found along the South African coast compares well with other Southern Hemisphere countries, such as Chile and New Zealand. The Southern Hemisphere has been suggested as the origin and centre of diversity for the Bangiales, but still remains relatively unexplored, and further investigations are likely to yield further species and species links.

PREFACE

I declare that this thesis is my own work and has not been submitted in this or any form to another university. Where use has been made of the research of others, it has been duly acknowledged in the text.

Work discussed in this thesis was carried out at the Department of Biological Sciences, University of Cape Town, South Africa under the supervision of Professor J.J. Bolton and A/Prof. R.J. Anderson and at the Department of Biology, Ghent University, Belgium under the supervision of Prof. O. De Clerck and Prof. F. Leliaert.

Signed: Signed by candidate **Name:** Miss M. M. Reddy **Date:** 16/02/2018

DECLARATION 1–PLAGIARISM

I, Mageshnee Mayshree Reddy declare that:

1. The research in this thesis, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other person's data, pictures, graphs, or other information unless specifically acknowledged as being sourced from other persons.

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a) Their words have been re-written but general information attributed to them has been referenced.

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DECLARATION 2– PUBLICATIONS AND CONFERENCE PROCEEDINGS

Publication

Details of Chapter 2 have been published:

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Conferences

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Year: 2017

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Contribution: Lead author and presenter

Co-Authors: O. De Clerck, R.J. Anderson, F. Leliaert, J.J. Bolton

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Contribution: Lead author and presenter

Co-Authors: R.J. Anderson, J.J. Bolton

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CHAPTER 1

General Introduction

1.1. THE BANGIALES

The Bangiales is an order of the Rhodophyta and comprises a variety of seaweeds. The structurally simple Bangiales belong to the class Bangiophyceae and are placed sister to the more complex Florideophyceae (Saunders & Hommersand, 2004; Milstein & Oliveira, 2005; Sutherland *et al*., 2011; Kucera & Saunders, 2012). The Bangiales are found throughout the world (Sutherland *et al*., 2011) and are best known for their commercial value in the nori industry (Yang *et al*., 2017a).

The taxonomy of the Bangiales has been notoriously difficult due to limited morphological characters for identification. The order initially comprised two genera based on morphology, *Porphyra* (bladed) and *Bangia* (filamentous). An additional bladed genus, *Miuraea* (Kikuchi *et al*., 2010) and three filamentous genera, *Dione*, *Minerva* and *Pseudobangia*, were later added to the order (Müller *et al*., 2005; Nelson *et al*., 2005). More recently a taxonomic revision of the Bangiales based on molecular sequences showed that diversity in this order was vastly underestimated and a total of fifteen genera was recognised (Sutherland *et al*., 2011). This included four new bladed genera that were described (*Boreophyllum*, *Clymene*, *Fuscifolium* & *Lysithea*) and two genera that were resurrected (*Pyropia* & *Wildemania*), in addition to the two previously described bladed genera, *Porphyra* (C. Agardh, 1824) and *Miuraea* (Kikuchi *et al*., 2010) (Sutherland *et al*., 2011). Three new filamentous genera were also recognized but not named (*Bangia* 1–3; Sutherland *et al*., 2011) in addition to the four previously described filamentous genera, *Bangia*, *Dione*, *Minerva* and *Pseudobangia*. Since then an additional bladed genus, *Neothemis* was discovered (Sánchez *et al*., 2014, 2015).

Throughout the thesis taxonomic changes following Sutherland *et al*. (2011) are adopted except where specified. The terms *Porphyra* sensu lato (bladed Bangiales) and *Bangia* sensu lato (filamentous Bangiales) are generally used when referring to studies prior to Sutherland's taxonomic revision or if the identity of a species is currently unknown according to the latest classification. To distinguish between the genera *Porphyra* and *Pyropia*, the abbreviation '*P*'. is used to indicate *Porphyra* and '*Py*'. to indicate *Pyropia*. The entire thesis is considered a single scholarly piece of work, for this reason species authorities are given at first mention in the thesis and are not repeated in subsequent chapters.

Socio-economic importance

The genus *Pyropia* (Bangiales) contains species that are among the most cultivated and economically valuable seaweeds worldwide (Mumford & Miura, 1988; Lim *et al*., 2017; Yang *et al*., 2017a). They have been traditionally eaten in Asia, Wales, Chile and New Zealand, and have become a popular food worldwide (Brodie *et al.*, 2008a). The longstanding traditional use of these algae makes the

bladed Bangiales a well-studied group, with over 250 years of research on them (Brodie *et al.*, 2008a). Commercially important species are cultivated in Asian countries, particularly in China, Japan and Korea (Niwa *et al*., 2005; Yang *et al*., 2017a), where this resource is estimated to be worth1.3 billion USD annually (Blouin *et al.* 2011). Other countries such as Britain, Canada, USA and Ireland continue to harvest wild stocks in small quantities (Blouin *et al.*, 2011). Three main species are cultivated for nori in Asia; *Py*. *yezoensis* (Ueda) M.S. Hwang & H.G. Choi, *Py*. *haitanensis* (T.J. Chang & B.F. Zheng) N. Kikuchi & M. Miyata and *Py*. *tenera* (Kjellman) N. Kikuchi, M. Miyata, M.S. Hwang & H.G. Choi. According to Niwa *et al*. (2005) *Pyropia tenera* is endangered in the wild in Japan and the continued cultivation of this alga in this region will rely on cultured strains.

The age of the Bangiales

The Bangiales is presumed to be an ancient order. Fossil evidence implies that the ancestor of the Bangiales can be traced back to just over a billion years ago, 1.05 (Gibson *et al*., 2017) –1.20 (Butterfield, 2000) billion years, representing the oldest taxonomically resolved eukaryote lineage with the earliest known form of sexual reproduction in multicellular organisms (Butterfield, 2000). This has been deduced from a discovery in Arctic Canada of fossilized filaments with a characteristic spore-producing phase that resembles extant *Bangia*. These fossilized filaments were ascribed to the order Bangiales and placed in a monotypic genus, *Bangiomorpha pubescens* N.J. Butterfield (Butterfield, 2000). Radioactive dating of surrounding volcanic rock constrains the age of the Bangiales to no older than *ca*. 1.2 BYA old and no younger than 800 MYA (Yang *et al*., 2016). Other Bangialean-like fossils discovered in China are believed to be no younger than 425–500 MYA (Campbell, 1980; Xiao, 1997). However, recent molecular studies suggest that the radioactive dating over-estimates the age of major red algal lineages (Berney & Pawlowski, 2006; Parfrey *et al*., 2011, Eme *et al*., 2014; Yang *et al*., 2016). An incorrect taxonomic assignment or errors in the fossil dates may account for these discrepancies (Eme *et al*., 2014; Yang *et al*., 2016). On the other hand, relaxed molecular clocks may not reflect such high rates of evolution prevalent in these algae (Eme *et al*., 2014). More recent molecular studies of the transcriptomics of *Porphyra umbilicalis* Kützing found that key genes presented an ancestral state (Brawley *et al*., 2017). This together with the ancestral appearance of reproductive characters, such as a pit plug with a cap but no membrane (Blouin *et al*., 2011) support an ancient origin of the Bangiales. Such a long evolutionary history might explain the incredibly high molecular diversity (Broom *et al*., 1999; Sutherland *et al*., 2011), deeply divergent genera (Sutherland *et al*., 2011) and a wide range of life history phases in the Bangiales (Blouin *et al*., 2011).

Distribution

The Bangiales have a cosmopolitan distribution but are more commonly found in temperate regions (Sutherland *et al*., 2011) and are remarkably successful in a range of habitats, predominantly in marine habitats but also rarely in freshwater and brackish water (Lüning, 1990). In marine habitats, species are commonly found in the eulittoral zone with some species dominating the uppermost intertidal and others found along the lower intertidal and subtidal. The Bangiales are also known to occur on various substrata such as rock (epilithically) and other hard substrata, marine animals (epizoically) and on other marine algae (epiphytically) (Sutherland *et al*., 2011).

Species occupying the intertidal are generally exposed to cyclic high light intensity, high temperature and desiccation. Desiccation can pose a threat to most marine organisms, but the high desiccation tolerance in some species of upper intertidal Bangiales allows these algae to occupy a unique niche, and makes them a leading candidate for studies on stress tolerance (Blouin *et al*., 2011; Brawley *et al*., 2017). Adaptations such as the production of Mycosporine-like Amino Acids (MAAs) which act as natural sunscreen, antioxidants, osmoregulators and the cyclic electron flow in Photosystem I activity may facilitate survival in environments that are otherwise hostile for most other marine organisms (Karsten & West, 2000; Blouin *et al*., 2011; Gao & Wang, 2013). The high desiccation tolerance of some species of the bladed Bangiales may contribute to their successful cultivation (Blouin *et al*., 2011).

Life history

Kathleen Drew (1949) was the first to demonstrate that *Conchocelis rosea* Batters represented a stage in the life history of *P. umbilicalis* rather than a different species. She referred to this stage as the conchocelis-phase. Studies on the conchocelis-phase of both filamentous and bladed Bangialean species worldwide followed (Graves, 1955; Tseng & Chang, 1955; Miura, 1961; Richardson & Dixon, 1968). Based on our present knowledge this phase appears to be common to species belonging to various genera in the order as delimited by Sutherland *et al*. (2011).

The conchocelis-phase is a shell-boring microscopic stage representing the diploid sporophytic phase of the heteromorphic life cycle of the Bangiales. The latter alternates with haploid gametophytes, which are the macroscopic thalli commonly observed on the seashore (Drew, 1949, 1954; Graves, 1955). Gametophytes may present a monoecious, dioecious, androdioecious (hermaphroditic) or protandric state and are able to reproduce sexually or asexually, in both the bladed and conchocelis-phase, via various types of spores (Drew, 1949, 1954; Graves, 1955; Nelson *et al*., 1999). Modes of reproduction may vary among species and may even differ within a single species in different regions. For example individuals of *P. umbilicalis* that occur in the northeastern Atlantic generally reproduce

sexually and are dioecious, while individuals of the same species in northwestern Atlantic generally reproduce asexually (Blouin *et al*., 2007, 2011).

1.1.1. The need for a taxonomic revision of the Bangiales

In the past, species could only be delimited based on a limited set of morpho-anatomical characters such as the morphology of the thallus, the structure of vegetative and reproductive cells, or a combination of these characters. However, the morphological simplicity of the group, with species forming monostromatic or distromatic filaments/blades led to much taxonomic confusion over the years (Sutherland *et al*., 2011). Life history traits (Kornmann, 1994; Brodie & Irvine, 1997; Holmes & Brodie, 2004) and habitat preference have additionally been used as distinguishing characters for some species of Japanese (Miyata & Kikuchi, 1997) and South African (Griffin *et al*., 1999a) bladed species, as well as for some filamentous species in the North Atlantic. Habitat preference is a readily distinguishable trait and allows for easy identification in the field. However, morphological homoplasy in the vast majority of Bangialean species limits the use of this character for many species and emphasizes the need for a more universal approach such as a molecular approach (Broom *et al*., 1999).

Exploring potential molecular markers and methods for a molecular taxonomic approach

One of the first molecular markers used for the identification of species in *Porphyra* was isozyme electrophoresis (Lindstrom & Cole, 1990, 1992a, b, c; Lindstrom, 1993; Griffin *et al*., 1999a). This was followed by modern-day Sanger sequencing where the nSSU gene (nuclear), the RuBisCo spacer (chloroplast) and later, the *rbc*L gene (chloroplast), have been largely favoured (Oliveira *et al*., 1995; Brodie *et al*., 1996, 1998; Müller *et al*., 1998; Broom *et al*., 2002; Klein *et al*., 2003; Lindstrom & Fredericq, 2003; Nelson *et al*., 2005; Brodie *et al*., 2008a; Milstein *et al*., 2012).

The nuclear encoded nSSU is a slow-evolving gene used to resolve deep nodes, although parts of the gene may be more variable and have been used to resolve interspecies relationships (Broom *et al.*, 1999; Jones *et al*., 2004). However, this gene is more commonly used to make inferences about ancient speciation events, and to construct hypotheses on species radiation and distribution over a long evolutionary scale (Nelson *et al.*, 2006; Brodie *et al.*, 2008a; Xu *et al*., 2017). Some of the limitations associated with the nSSU gene, are indels or Group I introns that may be present in this gene, making sequence alignment difficult (Müller *et al*., 2001; Teasdale *et al*., 2009; Sutherland *et al.*, 2011). The chloroplastic *rbc*L gene, which is faster evolving than the nSSU gene, has been commonly used to resolve generic and species level differences in a number of Bangiales. However, this gene has limited resolution when distinguishing between closely related or recently diverging species (Brodie *et al.*, 2008a, b; Sutherland *et al*., 2011; Guillemin *et al*., 2016). The mitochondrial

encoded DNA barcoding gene, *cox*1, which is even faster evolving than the *rbc*L gene, has been shown to effectively distinguish between species (Robba *et al*., 2006; Kucera & Saunders, 2012), including recently diverging species (Guillemin *et al*., 2016) as well as being able to recognize cryptic diversity (Kucera & Saunders, 2012) in the bladed Bangiales. This gene however, may contain introns and will require optimisation of routinely used primers (Saunders & Moore, 2013) or the design of new primers. Lastly, because this gene is mitochondrially inherited it is unable to detect hybridisation and introgression ((Mols-Mortensen *et al*., 2012).

The limitations associated with the use of a single marker are well documented (Leliaert *et al*., 2014 and references therein), and therefore a combination of independently evolving loci is generally favoured. This will provide more reliable results as species trees can be obscured due to gene tree incongruence. A combination of the nSSU and *rbc*L has traditionally been the most commonly used pair of markers for studies on the Bangiales (Müller *et al*., 1998; Broom *et al*., 2002; Klein *et al*., 2003; Lindstrom & Fredericq, 2003; Nelson *et al*., 2005; Brodie *et al*., 2008a; Milstein *et al*., 2012). However, many recent studies have used a combination of the *rbc*L and *cox*1 genes (Kucera & Saunders, 2012; Mols-Mortensen *et al*., 2012; Xie *et al*., 2015; Guillemin *et al*., 2016). The varying combination of markers sometimes make comparisons among species difficult because the *rbc*L gene (common to both combinations) may not always distinguish between closely related species (Brodie *et al*., 2008a, b; Guillemin *et al*., 2016). Nevertheless, the application of independently evolving loci has resulted in the recognition of increased species diversity and has resolved many taxonomic relationships in the Bangiales (Sutherland *et al*., 2011).

More recent studies have applied a set of analytical tools to various unlinked loci in order to reduce the subjectivity that is often associating with defining species boundaries. Three commonly applied methods are; the Automatic Barcode Gap Discovery (ABGD), General Mixed Yule Coalescent (GYMC) and Poisson Tree Processes (PTP). These methods have recently been applied to the Bangiales and produced promising results (Guillemin *et al*., 2016).

Taxonomic revision of the Bangiales based on molecular data

Initial molecular phylogenies of the Bangiales indicated that the generic division based on morphology did not reflect the evolutionary history of the order because the filamentous and bladed forms were polyphyletic (Oliveira *et al*., 1995; Müller *et al*., 1998). Based on these findings, two outcomes were possible. The first was to merge the bladed and filamentous forms into a single genus, making the Bangiales a monotypic order. In this case the name *Bangia* (type of the order) would be conserved because it is the type genus in the order. The second was to recognize each well-supported clade as a separate genus, resulting in several genera instead of two (Oliveira *et al*., 1995). In 2011,

the latter approach was adopted and used to the resolve the taxonomy of the Bangiales (Sutherland *et al*., 2011).

Until 2005 only two genera were recognized in the Bangiales, but since then three additional filamentous genera, *Pseudobangia* (Müller *et al*., 2005), *Dione* and *Minerva* (Nelson *et al*., 2005), were described and all currently represent monotypic genera. *Dione* and *Minerva* are ancestral genera in the order and together with the bladed genus, *Lysithea*, are endemic to New Zealand (Sutherland *et al*., 2011). Seven of the 15 genera recognized by Sutherland *et al*. (2011) are present in this region, where high species diversity and deeply divergent species can be found (Broom *et al*., 1999). This supports a Southern Hemisphere (eastern Gondwanaland) origin and centre of diversity for the order (Broom *et al*., 2004). However, based on our present knowledge this does not strictly apply to individual genera, as some appear to be ancestral and widespread in the Northern Hemisphere, such as *Pyropia* (Sutherland *et al*., 2011; Xu *et al*., 2017).

Due to the morphologically cryptic nature of the Bangiales, a much-needed taxonomic revision was conducted using the nuclear encoded nSSU gene and the chloroplastic *rbc*L gene, and applied to taxa from around the globe. The study identified an additional seven new genera (Sutherland *et al*., 2011). Sutherland *et al*. (2011) as well as highlighted the need for regional biodiversity assessments, particularly in understudied regions, and suggested that many more species or genera probably remain undiscovered. In accordance, an additional bladed genus, *Neothemis* was discovered in the Mediterranean (Sánchez *et al*., 2014, 2015).

In summary, the monotypic Bangiales consists of a single family which includes an extinct filamentous genus (Butterfield, 2000) and 17 extant genera, of which nine are bladed and seven are filamentous (Sutherland *et al*., 2011; Sánchez *et al*., 2014, 2015). Almost half of all species recognized in the bladed genera are undescribed species or represent species identified with some level of uncertainty (Sutherland *et al*., 2011). These species (identified based only on molecular data) have been assigned codes, e.g. GRB or ROS (Sutherland *et al*., 2011), and many have not yet been formally described. This is understandable as matching names with clades in the Bangiales is time consuming and can be difficult. However, the same naming convention has been adopted to denote species that represent morphologically cryptic taxa (Niwa *et al*., 2014; López-Vivas *et al*., 2015) and which cannot be named following conventional taxonomy. Bangialean species assigned codes could therefore represent those that have not been subjected to morphological or anatomical analyses (Jones *et al*., 2004; Guillemin *et al*., 2016), or cryptic species.

Implications of the taxonomic revision and a new look at individual genera in the Bangiales

One of the major consequences of the taxonomic revision (Sutherland *et al*., 2011) from a socio-economic point of view is that commercially important species, which have been cultivated in Asia for thousands of years, now belong to the genus *Pyropia* and not *Porphyra* as they once did (Zuccarello, 2011). The reassignment of species into different genera meant that genera previously known to be diverse, such as '*Porphyra*' and '*Bangia*', now contain far fewer species. On the other hand, the vast majority of species in the order are now contained in the less well-known resurrected genus *Pyropia*.

The genus *Pyropia* was erected by J. Agardh in 1889 and the type, *Pyropia californica* J. Agardh (now *Py. nereocystis* (C.L. Anderson) S.C. Lindstrom), was described but was later merged with *Porphyra*. In 2011, the genus was resurrected by Sutherland *et al*. (2011) and at present *Pyropia* is the most speciose, economically valuable, widely distributed and morphologically variable genus in the Bangiales. It consists of 87 species that are currently taxonomically accepted according to Lim *et al*. (2017) and additionally includes numerous molecular species (undescribed species). However, this could be underestimated given the rapid addition of species of *Pyropia*. In this genus many more species are found in the Northern compared to the Southern Hemisphere, but this could reflect a lack of research in the Southern Hemisphere rather than the evolutionary history of *Pyropia*. Some Asiatic and Mediterranean species belonging to this genus have been widely introduced around the world and their origins can probably be traced to the aquaculture and export of certain species. These include, *Py*. s*uborbiculata* (Kjellman) J.E. Sutherland, H.G. Choi, M.S. Hwang & W.A. Nelson (Broom *et al*., 2002; Monotilla & Notoya, 2004; Milstein & Oliveira, 2005; Tsutsui *et al*., 2005; Neefus *et al*., 2008;Vergés *et al*., 2013a), *Py. leucosticta* (Thuret) Neefus & J. Brodie (although the taxonomy of this species remains dubious; Brodie *et al*., 2008a; Mols-Morstensen *et al*., 2012) and *Py. yezoensis*, all of which are now introduced in the North Atlantic (Neefus *et al*., 2008). Incidentally, the commercially important *Py. yezoensis* is less widespread than the diminutive, *Py*. s*uborbiculata*. The small size of the latter may facilitate its distribution via human-mediated transport and probably makes it more difficult to detect once introduced. Alternatively aspects of the life cycle or niche occupied by *Py*. s*uborbiculata* might explain why this alga is so widespread.

Porphyra sensu stricto was formerly known as a speciose genus with species widely distributed from the tropics to the poles (Sutherland *et al*., 2011), comprising 64 currently taxonomically accepted species names (Lim *et al*., 2017). Only eight of these species names have been confirmed to represent distinct species and can be attached to molecular information. Additionally *ca*. 20–30 molecular species/entities have been identified or confirmed to belong to *Porphyra*, but have not yet been named. The identities of the remaining species names are yet to be placed in a molecular phylogeny

according to the most recent classification (Sutherland *et al*., 2011). Some species names that are currently taxonomically accepted have not yet been tested molecularly and include locally abundant or common species assigned to '*Porphyra*' based on morphological analyses, but many of these have not yet been sequenced and could belong to new or different genera. For example, the common intertidal '*Porphyra*' (misidentified as *P. columbina* Montagne) in New Zealand was found to be a new species of *Pyropia* based on molecular (Broom *et al*., 1999; Nelson, 2010) and subsequent morpho-anatomical analyses (Nelson, 2013). Furthermore, for species confirmed to belong to the genus *Porphyra*, many rare species may be hidden amongst the common names. Nevertheless, based on our current understanding *Porphyra* now represents the second most speciose genus in the Bangiales, based on sequence data. The type, *P. purpurea* (Roth) C. Agardh was first described from the Northern Hemisphere where five other species occur; *P. umbilicalis*, *P. linearis* Greville, *P. dioica* J. Brodie & L.M. Irvine, *P. corallicola* H. Kucera & G.W. Saunders and *P. mumfordii* S.C. Lindstrom & K.M. Cole. Far fewer species have been described from the Southern Hemisphere, and only two have sequence information attached to them. These are *P. capensis* Kützing from southern Africa (although this appears to consist of a species complex; Jones *et al*. 2004) and *P. lucasii* Levring from Australia.

It is noteworthy that *P. purpurea* (type) is deeply divergent from other species included in the *Porphyra* clade and is recovered as a sister taxon to all other species of *Porphyra* under Maximum Likelihood analyses (Sutherland *et al*., 2011). Further sampling and species discovery may identify additional taxa that may clarify the relationship between *P. purpurea* and all other species presently assigned to the *Porphyra* clade. If so, it is anticipated that future studies may restrict the genus *Porphyra* to species belonging to the '*P. purpurea* clade'. All other currently recognized *Porphyra* species will then belong to a yet undescribed genus.

The second resurrected bladed genus *Wildemania* (Sutherland *et al*., 2011), and the generitype *Wildemania amplissima* (Kjellman) Foslie were first described by De Toni in 1809 based on a specimen originally identified by Kjellman. Species in this genus are distinguished by their occurrence in the low subtidal, their reddish pink colour and primarily distromatic blades (however *W. amplissima* is monostromatic; Brodie & Irvine, 2003). *Fuscifolium* is the only other bladed genus known to include species that are distromatic. According to Sutherland *et al*. (2011) *Wildemania* lacked nodal support as a distinct genus using only the nSSU gene, but was supported by the *rbc*L gene alone and a combined *rbc*L and nSSU analysis. A complete mtDNA genome analysis has since confirmed the phylogenetic position and distinctness of this genus (Hughey, 2016; Silva & Hughey, 2016). *Wildemania* contains eight taxonomically accepted species and five molecular species. Most species have been recorded from the Northern Hemisphere (America & Asia) and only *W. miniata* (C. Agardh) Foslie is known from the Southern Hemisphere based on morphological identification

(Ramírez & Santelices, 1991). However, this may have been a misidentification of *W. amplissima* or a species that superficially resembles *W. miniata* and thus the occurrence of *W. miniata* in Chile remains to be confirmed using molecular data. Indeed, a new molecular species endemic to Chile has been recently added to this genus (Guillemin *et al*., 2016) but was not identified as *W. miniata*.

The remaining six bladed genera, *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Miuraea* and *Neothemis* include no more than five species each and species generally appear to be regionally confined. *Miuraea* (North America & Asia), *Clymene* (Australia & NZ) and *Lysithea* (Subantarctic islands south of NZ) are monotypic genera. *Fuscifolium* and *Neothemis* contain two described species each and the former includes one molecular species (Sutherland *et al*., 2011; Sánchez *et al*., 2014, 2015; Guillemin *et al*., 2016) and *Boreophyllum* contains five described species, two of which were recently discovered (Lindstrom *et al*., 2015b).

Bangia sensu lato previously consisted of 102 species and 46 infraspecific names, only 16 of which are currently taxonomically accepted names, and only five (*B. atropurpurea* (Mertens ex Roth) C. Agardh, *B. fuscopurpurea* (Dillwyn) Lyngbye, *B. gloiopeltidicola* Tanaka, *B. vermicularis* Harvey and *B. maxima* Gardner) of these have sequence information. *Bangia* sensu stricto now refers to the monotypic freshwater genus containing *B. atropurpurea*. The marine, *Bangia fuscopurpurea* appears to be polyphyletic (Sutherland *et al*., 2011) which suggests that the name has been misapplied, or that this species consists of multiple species. The freshwater *Bangia atropurpurea* and the marine species *Bangia fuscopurpurea* were once considered a single species, but have been shown to represent distinct species (Müller *et al*., 2003). *Pseudobangia*, *Dione* and *Minerva* are all monotypic genera, while the three unnamed genera, *Bangia* 1–3, are diverse and widespread (Sutherland *et al*., 2011). For example, *Bangia* 1 contains species/molecular species from various regions in the Northern and Southern Hemispheres (Sutherland *et al*., 2011). The filamentous Bangiales are known to include a number of cryptic species (Bödeker *et al*., 2008) and research from around the globe is continually adding to the diversity of this group.

An update on the Bangiales since the taxonomic revision in 2011

A new bladed genus was discovered in the western Mediterranean, but the proposed name, *Themis* (Sánchez *et al*., 2014) and subsequent name change, *Neothemis* (Sánchez *et al*., 2015) are both invalid and so the genus, and two species included in it, currently remain without valid names (Wynne & Schneider, 2016).

Since the taxonomic revision by Sutherland *et al*. (2011) and the application of molecular techniques in studies of the Bangiales, there has been a steady increase in the number of new species recognised, globally. These include several new species of *Pyropia*: *Py. orbicularis* M.E. Ramírez, L. Contreras

Porcia & M.-L. Guillemin (Ramírez *et al*., 2014) from Chile; *Py. peggicovensis* H. Kucera & G.W. Saunders (Kucera & Saunders, 2012) from Nova Scotia, Canada; *Py. njordii* Mols-Mortensen, J. Brodie & Neefus (Mols-Mortensen *et al*., 2012), *P. spatulata* T. Bray, A.C Mathieson & C. Neefus, *P. novaeangliae* A.C. Mathieson, T. Bray & C. Neefus, *P. collinsii* C. Neefus, A.C. Mathieson & T. Bray and *P. stamfordensis* C. Neefus, T. Bray & A.C Mathieson (Bray, 2006; unpublished, Mathieson & Dawes, 2017) from the North Atlantic; *Py. raulaguilarii* Mateo-Cid, Mendoza-González & Sentíes (Mateo-Cid *et al*., 2012) from the Pacific coast of Mexico; *Py. parva* A. Vergés & N. Sánchez (Sánchez *et al*., 2014) from the western Mediterranean, *Py. nitida* L.K. Harden, K.M. Morales & Hughey (Harden *et al*., 2016), *Pyropia columbiensis* S.C. Lindstrom (Lindstrom *et al*., 2015a) and *Pyropia protolanceolata* S.C. Lindstrom & J.R. Hughey (Lindstrom *et al*., 2015a) from the Pacific coast of America. Relatively fewer species of *Boreophyllum* were discovered, and include *B. aleuticum* S.C. Lindstrom & M.R. Lindeberg and *B. ambiguum* S.C. Lindstrom (Lindstrom *et al*., 2015b) from the North Pacific. Two new species in the new genus *Neothemis*, *N. iberica* (A.Vergés & N. Sánchez) A. Vergés & N. Sánchez and *N. ballesterosii* (A.Vergés & N. Sánchez) A. Vergés & N. Sánchez (Sánchez *et al*., 2014) from the western Mediterranean were also added to the Bangialean flora, however see an earlier comment about the invalid names. Lastly, only one new species of *Porphyra* has been described since Sutherland *et al*. (2011), this is *P. corallicola* (Kucera & Saunders, 2012), a species associated with crustose coralline algae in the North Atlantic.

Some species that were initially identified molecularly and assigned a unique code have now been described following additional morpho-anatomical analyses. These include a common intertidal species in New Zealand, ROS54 (Broom *et al*., 1999) now described and named *Py. plicata* W.A. Nelson (Nelson, 2013). Another molecular species from this region, PTK was named *Py. francisii* W.A. Nelson & R. D'Archino (Nelson & D'Archino, 2014). On the other hand, a few molecular species assigned unique codes were found to be conspecific with existing species, such as GEP = *Py. koreana* (M.S. Hwang & I.K. Lee) M.S. Hwang, H.G. Choi Y.S. Oh & I.K. Lee (Nelson *et al*., 2014), Piaui = *Py. vietnamensis* (Tak. Tanaka & Pham-Hoàng Ho) J.E. Sutherland & Monotilla (Milstein *et al*., 2015) and P2 = *Py. kurogii* (S.C. Lindstrom) S.C. Lindstrom. Other species names that were synonymised are as follows, *Porphyra drewiana* Coll & E.C. Oliveira = *Pyropia spiralis* (E.C. Oliveira & Coll) M.C. Oliveira, D. Milstein & E.C. Oliveira (Milstein *et al*., 2012), *Py. olivii* Orfanidis, Neefus & Bray = *Py. koreana* (M.S. Hwang & I.K. Lee) M.S. Hwang, H.G. Choi Y.S. Oh & I.K. Lee (Vergés *et al*., 2013b) and *Py. rosengurttii* J. Coll & J. Cox = *Py. elongata* (Kylin) Neefus & J. Brodie.

Additionally, a number of new molecular species have been discovered, and in accordance with previous convention have been assigned codes. Many of these molecular species have been used in later Bangialean phylogenies (Mols-Mortensen *et al*., 2012, 2014, Kucera & Saunders, 2012) to

resolve species relationships despite not being named. New molecular species discovered have been assigned code and are as follows; from Chile and the Falkland Islands: *Porphyra* CHB-F, FIH, FIG; *Wildemania* FI; *Fuscifolium* CHA and *Pyropia* CHG-K, FID, FIA (Jones *et al*., 2004; Broom *et al*., 2010; Guillemin *et al*., 2016); North America: *Bangia* sp. 1BAN, 2BAN, *Wildemania* sp. 5POR, *Pyropia* sp. 2Cal and *Pyropia* sp. 6POR (Kucera & Saunders, 2012), one molecular species from the North Atlantic (Faroe Islands) *Porphyra* sp. FO (Mols-Mortensen *et al*., 2012) and more recently five new cryptic species from China, *Pyropia* sp. 1–5 (Yang *et al*., 2017b). Further investigations of morpho-anatomical or ecologically distinguishing traits in many of these molecular species are predicted to result in more species being described.

As previously mentioned, molecular species that are assigned codes could signify species that are morphologically cryptic. For example, López-Vivas *et al*. (2015) identified two morphologically cryptic species in the complex *Py*. *hollenbergii* (E.Y. Dawson) J.E. Sutherland, L.E. Aguilar Rosas & R. Aguilar-Rosas which they referred to as GCI and GCII, as well as a cryptic species similar in morphology to *Py*. *pendula* (E.Y. Daywson) J.E. Sutherland, L.E. Aguilar-Rosas & R. Aguilar-Rosa which they referred to as CGIII. Conversely, some species that were previously considered cryptic were found to be morphologically distinct and have been described, such as *Py*. 523 (Sutherland *et al*., 2011) = 1POR (Kucera & Saunders, 2012) = *P*. unknown #1 (Lindstrom, 2008) = now *Py*. *unabbottiae* S.C. Lindstrom (Lindstrom *et al*., 2015b); *Py*. FAL (Sutherland *et al*., 2011) = *Py*. MIG (Sutherland *et al*., 2011) = now *Py*. *bajacaliforniensis* Aguilar-Rosas & Hughey (Lindstrom *et al*., 2015a); 1Cal (Kucera & Saunders, 2012) = now *Py*. *montereyensis* S.C. Lindstrom & Hughey (Lindstrom *et al*., 2015a); *Porphyra sp. Unknown #2* (Lindstrom, 2008) = now *Pyropia taeniata* S.C. Lindstrom (Lindstrom *et al*., 2015b) and *Porphyra* Unknown #4 (Lindstrom, 2008) = now *Boreophyllum aleuticum* S.C. Lindstrom & M.R. Lindeberg (Lindstrom *et al*., 2015b).

Understandably, the taxonomic revision by Sutherland *et al*. (2011) could not include all known species or attempt to sequence all type specimens assigned to the Bangiales. Therefore, many type specimens will need to be re-investigated and classified using the currently accepted generic division for the order (Sutherland *et al*., 2011). This may further result in a) newly described species being synonymized with existing names, b) the description of new species or combinations, or c) the confirmation of morphological species using a molecular approach. As an example, a fragment from the type specimen of *Py*. *pulchra* (Hollenberg) S.C. Lindstrom & Hughey was recently sequenced and showed that this species was conspecific with *Py*. *smithii* (Hollenberg & I.A Abbott) S.C. Lindstrom and these have been synonymized with the former name retained (Lindstrom & Hughey, 2016). Lastly, sequence data are available for additional species that were not included in the taxonomic revision by Sutherland *et al*. (2011) and some recent additions include *Py*. *elongata*, *Porphyra*

oligospermatangia C.K. Tseng & B.F. Zheng (needs to transferred to *Pyropia*) and *Pyropia thulaea* (Munda & P.M. Pedersen) Neefus (Mols-Mortensen *et al*., 2012, 2014).

Misapplied names and misleading distribution ranges

Historically (*ca*. 1800s) only a few species were recognized for *Porphyra* sensu lato; because of the morphological simplicity of these species. This meant that common names were widely misapplied and perpetuated the idea of widely distributed species in the Bangiales. As an example, the apparent global distribution of species of *Porphyra* identified using morphology alone reveals that some species appear to be widely distributed, such as *P. umbilicalis*, *P. linearis* and *P. capensis* (Fig. 1.1). The name *P. umbilicalis* has been widely applied to taxa in the Northern and Southern Hemispheres according to several authors (Broom *et al*., 1999; Brodie *et al*., 2008b; Guillemin *et al*., 2016), and accordingly this species was erroneously thought to be widespread (Fig. 1.1). However, molecular evidence suggests that *P. umbilicalis* is indeed confined to the northwestern and northeastern Atlantic (Brodie *et al*., 2008a, b). Inaccurate taxonomy therefore perpetuates the idea of widely distributed or cosmopolitan species of *Porphyra* (Fig. 1.2). This concept is increasingly being challenged and shown not to hold true for many species not only in *Porphyra* but also in other genera of the Bangiales (Brodie *et al*., 2008b; Guillemin *et al*., 2016), as well as for other seaweeds (Won *et al*. 2009, 2010; Payo *et al*., 2013; Vieira *et al*., 2014).

Similarly, *Py. columbina* has been documented as a) morphologically diverse, b) found in a variety of habitats and is c) geographically widespread in the Southern Hemisphere (Ramírez & Santelices, 1991). The name has been widely applied to common or locally abundant species of '*Porphyra*' in New Zealand, Chile and some Subantarctic Islands (e.g. Falkland Islands). In both continental regions, the name has concealed unique and endemic species, such as *Py. plicata* (Nelson, 2013) in New Zealand, *Py. orbicularis* in Chile (Ramírez *et al*., 2014) and numerous other molecular species (Guillemin *et al*., 2016). Based on molecular data, *Py. columbina* is now thought to be restricted to the Subantarctic Islands (Nelson & Broom, 2010).

Fig. 1.1. Map showing the generalized (extrapolated) distribution of species in the genus *Porphyra* based on morphological identification.

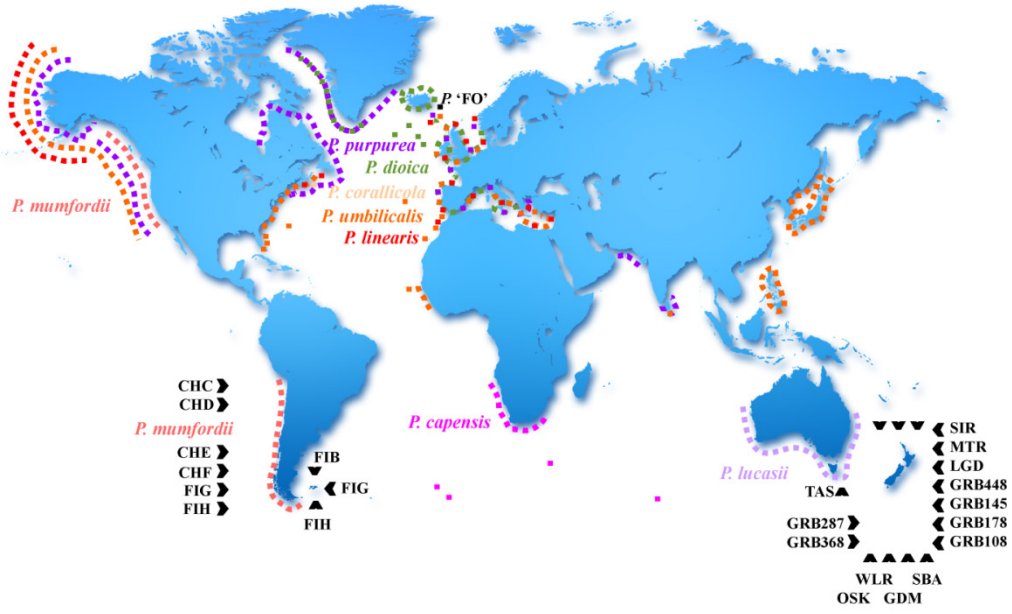

Fig. 1.2. Map showing the generalized (extrapolated) distribution of species in the genus *Porphyra* confirmed using molecular identification including new molecular species.

Note that Figs. 1.1 and 1.2 are based on generalized (extrapolated) distributions of species and are by no means geographically accurate. For example if a species was recorded in Chile, its distribution is extrapolated for the entire country even though it might only occur at a few sites. Extrapolated distributions were used due to a lack of detailed geographic information for species records. The purpose of these figures are intended to illustrate how misidentification based on morphology masks species diversity and perpetuates the idea of widely distributed species.

Although many species in the Bangiales have been found to be regionally confined (Brodie *et al*., 2008b; Lindstrom *et al*., 2015b; Guillemin *et al*., 2016), many others have been found to have a wider distribution range than previously thought. For example, *Boreophyllum birdiae* (Neefus & A.C. Mathieson) Neefus, first recorded in North America, has been shown to occur in Iceland (Mols Mortensen *et al*., 2012) and *Miuraea migitae* (N. Kikuchi, S. Arai, G. Yoshida & J.A. Shin) N. Kikuchi, S. Arai, G. Yoshida, J.A. Shin & M. Miyata, first described from Japan (Kikuchi *et al*., 2010), and is now known from Korea (Koh *et al*., 2016). Three more Japanese species, *Py. kinositae* (Yamada & Tak. Tanaka) N. Kikuchi, M. Miyata, M.S. Hwang & H.G. Choi, *Py. pseudolinearis* (Ueda) N. Kikuchi, M. Miyata, M.S. Hwang & H.G. Choi and *Py. tanegashimensis* (Shinmura) N. Kikuchi & E. Fujiyoshi are now known from China (Yang *et al*., 20107b) and the latter is also present in the Phillipines (Dumilag & Aguinaldo, 2017). Most notably, *P. mumfordii*, which was first recorded in North America and Canada, has recently been found along the Chilean coast and is the only known species of *Porphyra* (based on sequence data) to traverse both Hemispheres. In addition to new distribution records, some Asiatic species have been found to be introduced in parts of America (*Py. katadae*, *Py. suborbiculata*, *Py. yezoensis*; Neefus *et al*., 2008) and New Zealand (*Py. koreana*; Nelson *et al*., 2014), and the widely introduced *Py. suborbiculata* has been recorded in various regions globally, making this species truly widespread (Broom *et al*., 2002; Monotilla & Notoya, 2004; Milstein & Oliveira, 2005; Tsutsui *et al*., 2005; Neefus *et al*., 2008; Vergés *et al*., 2013a).

The above examples demonstrate how the application of molecular techniques has helped clarify many regional floras (Mols-Mortensen *et al*., 2012; Xie *et al*., 2015; Guillemin *et al*., 2016; Dumilag *et al*., 2016; Dumilag & Aguinaldo, 2017; Yang *et al*., 2017b), however many regions remain to be assessed, particularly in the Southern Hemisphere such as in South Africa.

1.1.2. The history and taxonomy of the Bangiales in South Africa

Two species of *Porphyra* were first described from South Africa in 1843, *P. capensis* Kützing and *P. augustinae* nom. illeg. (Kützing, 1843). However, J. Agardh considered the latter to be a developmental stage rather than a different species and the two species were synonymized, with the name *P. capensis* conserved over *P. augustinae* nom. illeg. (Agardh, 1883). Thereafter, two additional

species, *P. vulgaris* C. Agardh nom. illeg. and *P. laciniata* var. *capensis* (Kützing) Grunow, were recorded (Delf & Michell, 1921) but these were similarly considered variations of *P. capensis* (Isaac, 1957; Graves, 1969; Seagrief, 1984; Silva *et al.*, 1996). Below is a description of *P. capensis* adapted from Stegenga *et al*., (1997) and Kützing (1843).

Porphyra capensis (Kützing, 1843)

Plants membranous, varying from light yellow through dark red /purple to almost black, varying in size from a few cm to over a metre. Thallus varying shape from orbicular to elongate; blade usually 100–150 µm thick. Cells in surface view rather irregularly arranged, rounded, 8–15 µm in diameter. Cells in cross section elongated, up to four times as long as broad, containing 1–2 stellate chloroplasts, each with a pyrenoid. Monoecious, dioecious or androdioecious with spermatangial areas visible around margins as pale or yellow band and carposporangial areas a darker red than vegetative areas (Fig. 1.3). Reproductive cells a similar shape to vegetative cells. Mature spermatangia containing up to 24 tiers of spermatia; carposporangia containing 32 carpospores in 8 tiers.

Fig. 1.3. *Porphyra capensis* showing an outer edge of reproductive tissue (female sori: pinkish-purple and male sori: yellow). Photo credit: Dave Dyer.

South Africa has a short history (1965–1977) of exporting wild stocks of '*P. capensis*' to the nori industry in Japan and is the only seaweed that has been exported from South Africa for human consumption (Anderson *et al*., 1989). However, due to its unfavourable characteristics, such as a thick

blade, export of this resource ceased. Nevertheless '*Porphyra. capensis*' is considered to be ecologically important throughout temperate southern Africa (South Africa & Namibia), and where it is locally dominant it provides food, shelter and habitat for various other marine organisms.

In 1997, Stegenga *et al.* recorded an additional species of *Porphyra* from South Africa, *P. gardneri* (G.M. Smith & Hollenberg) M.W. Hawkes and described two others: *P. saldanhae* Stegenga, Bolton & R.J. Anderson as well as an unnamed epiphytic species, *P.* sp. indet. *P. gardneri* and *P. saldanhae* have been transferred to the genus *Pyropia*. The epiphytic species *P.* sp. indet. was described based on morphology and has not been found again since its description, and therefore the genus to which it currently belongs remains to be confirmed. In 1999 an additional epiphytic species (albeit found growing on a different host), *P. aeodis* N.J. Griffin, Bolton & Anderson, was described by Griffin *et al.* (1999a). This species has also been transferred to the genus *Pyropia*. Griffin *et al*. (1999a) were the first to use molecular evidence (isozymes), to delimit bangialean species in South Africa.

Until 2004, a total of five species were recognised (based on morphological characters), of which four had been named and one remains unnamed. However, this count appears to be a gross underestimate using genetic methods (Jones *et al.*, 2004; Sutherland *et al.*, 2011). A preliminary assessment of the '*Porphyra*' (bladed Bangiales) species along the South African coast using the nSSU gene and a variable portion in that gene, V9, identified a total of 11 molecular entities (Jones *et al.*, 2004). This included two species that have been transferred to *Pyropia*, *Py. saldanhae* and *Py. aeodis*, as well as an unknown species *Py*. ZLI (Jones *et al.*, 2004; Sutherland *et al.*, 2011). Species of *Pyropia* and *Porphyra* from South Africa are distinguishable based on morpho-anatomical characters (Stegenga *et al*., 1997; Griffin *et al*., 1999a; Sutherland *et al*., 2011). However, it is unknown if species/molecular entities of *Porphyra* in South Africa differ morphologically. It is also unknown if *P. capensis* can be attached to any of these molecular species or if morpho-anatomical characters of all molecular entities are within the range of the current morphological description of *P. capensis*. For this reason *P. capensis* is referred to as *P. capensis* sensu lato which collectively refers to species of South African *Porphyra* in general.

The distribution of South African Bangiales

The distribution of *P. capensis* sensu lato along the South African coast extends from Port St. Johns on the east coast to Port Nolloth on the west coast (Fig. 2.1). Although first described from South Africa, *P. capensis* sensu lato has been recorded extending into Namibia (Graves, 1969; Seagrief, 1984; Stegenga *et al.*, 1997; Lluch, 2002) and further north into Angola (John *et al*., 1979, 2004). *Porphyra capensis* sensu lato has even been recorded further afield in the Southern Hemisphere, on the coasts of South America (Pujals, 1963; Ramírez & Santelices, 1991), and on various Southern Ocean Islands such as Tristan da Cunha, Gough Island and the Falkland Islands as well as other

Subantarctic Islands (Papenfuss, 1964; Chamberlain, 1965; Silva *et al*., 1996; Guiry & Guiry, 2018). However, the occurrence of *Porphyra capensis* sensu lato outside South Africa remains poorly understood and many distribution records based on morphology require DNA confirmation.

Along the South African coast the bladed Bangiales occur across two oceans and a transition zone between these regions, with each influenced by different current systems. The cool temperate west coast is influenced by upwelling from the Benguela Current, and the warm temperate Indian Ocean coasts on the south and east of South Africa are influenced by the warm, southward-flowing Agulhas Current. Biogeographic and genetic discontinuities have been found in species occurring across this region for a number of marine organisms (von der Heyden, 2009; Teske *et al.*, 2011a; Smit *et al*., 2017). Additionally, for '*P. capensis*' different forms are associated with different coasts along South Africa (Isaac, 1957; Graves, 1969).

South African bladed Bangiales are not only widely distributed geographically and highly variable in terms of morphology, but also exploit a wide range of habitats, ranging from substrata such as on rock (epilithic), marine animals (epizoic) and on other marine algae (epiphytic), especially on large brown algae, and they also display a wide variation in occurrence along the shore (tidal position) and reproductive strategy (Isaac, 1957; Graves, 1969; Maggie Reddy personal observation 2014–2018).

Current Bangiales species inventory for South Africa

To date seven valid bangialean species have been reported to occur in South Africa and four are likely endemic to southern Africa. These include the epilithic *Porphyra capensis* sensu lato and *Pyropia saldanhae*, and two epiphytic species, *Py. aeodis* and an unnamed *Porphyra* sp. indet. The other three species, *Py. gardneri*, *Py. suborbiculata* and *B. fuscopurpurea* are globally distributed. The identification of all globally distributed species has been based on morphological identification, and molecular evidence for these species are lacking.

1.2. RATIONALE AND MOTIVATION

Regional biodiversity assessments worldwide have revealed increased levels of diversity following the many taxonomic changes to the Bangiales. For example, in the North Atlantic 15 species of *Porphyra* were recognised using traditional taxonomy, seven of which occur in Canada. However, subsequent genetic analyses using COI-5P and *rbc*L markers revealed a total of 39 molecular species, 27 of which occur in Canada (Kucera & Saunders, 2012).

Similarly, in the Southern Hemisphere only a single species each of *Bangia* and *Porphyra* were known from the coasts of Australia and New Zealand. However, using a molecular approach studies

suggest at least 10 and 33 species (respectively) are likely to occur along these coastlines (Adams 1994; Broom *et al.*, 1999, 2002, 2004; Knight & Nelson, 1999; Nelson *et al*., 2001, 2006; Schweikert *et al.*, 2012). Broom *et al.* (1999) recorded 10 epilithic and five epiphytic species of *Porphyra* along the coast of New Zealand using a variable portion of the nSSU gene. Since then, a number of new and endemic species have been added to the New Zealand flora and some authors suggest that the already high species diversity recorded for New Zealand's bladed Bangiales (identifying 30–33 entities, 7–10 of which have been names and described) may not represent the true extent of diversity in this region (Broom *et al.*, 2002; Broom *et al.*, 2004; Nelson *et al.*, 2006; Sutherland *et al.*, 2011; Schweikert *et al.*, 2012).

Increased levels of diversity in the Bangiales have also been found in regions of the Southern Hemisphere that have been relatively poorly studied, such as in Chile and South Africa. In Chile, extensive species diversity and endemism have been discovered using various analytical species delimitation methods applied to molecular sequence data (*rbc*L & *cox*1; Guillemin *et al*., 2016). In South Africa, a preliminary genetic survey using the nSSU and V9 regions similarly suggested extensive species diversity along this coastline (Jones *et al.*, 2004). However, the preliminary biodiversity assessment by Jones *et al*. (2004) did not include specimens from throughout the distribution range of the Bangiales in South Africa, and only a single gene (nSSU) and a variable portion in that same gene (V9) were used to identify distinct molecular entities. These molecular entities might have represented distinct species but had not been subjected to morpho-anatomical studies.

1.3. RESEARCH QUESTIONS

CHAPTER 2: *How much biodiversity of the Bangiales is there in South Africa?*

Regional biodiversity assessments worldwide suggest high levels of undocumented species diversity in the Bangiales. A preliminary assessment of the South African coast supports this notion but further study is required.

Aim: To identify, quantify and place South African Bangiales species in an unambiguous molecular phylogeny.

Objective: Determine the total number of species and genera of the Bangiales present in South Africa. Genera were identified according to the classification of Sutherland *et al*. (2011) and species boundaries were defined based on a diverse collection of Bangiales throughout the known distribution range in South Africa. Three unlinked loci; the nuclear encoded nSSU, the chloroplastic *rbc*L and the

mtDNA *cox*1and various DNA-based species delimitation methods (ABGD, GMYC & PTP) were used for species delimitation.

CHAPTER 3: ***What is the diversity of the resurrected genus Pyropia in South Africa and how does this diversity relate to the rest of the world?***

The resurrected genus *Pyropia* (Sutherland *et al*., 2011) has never before been studied in South Africa although it has been included in a study of *Porphyra* sensu lato (Jones *et al*., 2004). *Pyropia* represents the most diverse and widespread genus in the Bangiales. Three species of *Pyropia* occur in South Africa, two of which have been previously assigned to the genus *Porphyra* but have since been reassigned to *Pyropia* based on molecular data (Jones *et al*., 2004; Sutherland *et al*., 2011). The third species has been identified as a new molecular entity but is currently lacking morpho-anatomical and ecological data. Additionally, two widely-distributed species (now belonging to the genus *Pyropia*) have been recorded in South Africa based on morphological identification but require confirmation using molecular data.

Aim: To re-assess, classify and describe any new species of *Pyropia* from South Africa, and re-examine specimens identified as the widely distributed *Py. gardneri* and *Py. suborbiculata* from this coastline. To document the known distribution ranges and contribute to the current understanding of all species of *Pyropia* in South Africa. In addition, to infer, from a multi-gene phylogeny, the phylogenetic relationships and genetic affinities of South African *Pyropia* relative to species from around the world.

Objective: Additional sequence data, morpho-anatomical data and ecological data were analysed for species of *Pyropia* to test for congruence of species boundaries identified in Chapter 2. A new species was described and key morpho-anatomical, ecological characters, as well as, distributions, were detailed for all species.

"*CHAPTER 4:** ***What is the diversity of the genus Porphyra in South Africa, how is it distributed and what are the mains drivers of diversity?".

The genus *Porphyra* traditionally consisted of a single species in South Africa, the morphologically variable and widespread *P. capensis* (Isaac, 1957; Graves, 1969). However, subsequent molecular studies suggest that there is a high level of diversity in this genus in South Africa (Jones *et al*., 2004). Additionally, the distribution of *P. capensis* sensu lato occurs across two oceans with contrasting environmental features and different morphological variants are known to occur in different oceans.

Aim: To identify, classify and describe any new species of *Porphyra* from South Africa using an integrative taxonomic approach. To determine if the different morphological variants represent different species. To further determine whether the occurrence of species in different oceans has impacted the genetic structure in this genus. Lastly, to propose evolutionary hypotheses that may explain genetic patterns.

Objective: Using a combination of markers such as the slowly evolving nSSU to resolve deep nodes and faster evolving markers such as the *rbc*L and *cox*1genes to aid in taxonomic and systematic inferences. Morphometric analysis was used to determine if species could be categorised using a combination of morpho-anatomical features that were consistent with molecular differences. In addition the faster evolving markers were used to make inferences on phylogeographic patterns and subsequent speciation of *Porphyra* in South Africa. Depending on the complexity of genetic patterns, paleoceanography and present day features of the nearshore were used to explain genetic patterns in this genus.

CHAPTER 2

A Rosette By Any Other Name: Species Diversity in the Bangiales (Rhodophyta) Along the South African Coast

2.1. INTRODUCTION

The Bangiales are morphologically simple red algae, widely distributed in the marine environment and to a lesser extent in brackish and fresh water. These algae are found from the tropics to the poles, but more commonly occur in temperate regions (Sutherland *et al*., 2011).

The order consists of one family, the Bangiaceae and was traditionally classified into two genera based on morphology, the bladed *Porphyra* and the filamentous *Bangia* (Engler, 1892; Garbary *et al*., 1980). However, early molecular phylogenetic data revealed that these two genera were polyphyletic (Oliveira *et al*., 1995; Müller *et al*., 1998; Broom *et al*., 1999). A re-examination of the Bangiales based on two molecular markers (the plastid, ribulose 1,5 bisphosphate carboxylase large subunit (*rbc*L) gene and the nuclear small subunit rRNA (nSSU) gene) applied to 157 taxa sampled worldwide and using type specimens where possible, revealed 15 well-supported clades. These were circumscribed, reinstated or supported as genera: the eight foliose genera *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Miuraea*, *Porphyra*, *Pyropia* and *Wildemania* and seven filamentous genera, four of which have been named (*Bangia*, *Dione*, *Minerva* & *Pseudobangia*) (Müller *et al*., 2005; Nelson *et al*., 2005; Sutherland *et al*., 2011). Consequently, several species of *Porphyra* and *Bangia* were transferred into new or resurrected genera and a number of undescribed species were highlighted (Sutherland *et al*., 2011). A ninth bladed genus, *Neothemis*, from the Mediterranean Sea was later added to the order (Sánchez *et al*., 2014, 2015). Molecular-assisted alpha taxonomy from a series of regional studies thereafter resulted in the recognition of many more (predominantly bladed) species (Kucera & Saunders, 2012; Mols-Mortensen *et al*., 2012; Mateo-Cid *et al*., 2012; Nelson, 2013; Nelson & D'Archino, 2014; Ramírez *et al*., 2014; Sánchez *et al*., 2014; Lindstrom *et al*., 2015a, 2015b; Guillemin *et al*., 2016).

The most routinely applied molecular markers for species delimitation in the bladed Bangiales are the nuclear encoded nSSU gene, the plastid encoded *rbc*L gene, and to a lesser extent the mitochondrial encoded DNA barcoding gene, cytochrome oxidase subunit 1 (*cox*1) (Robba *et al*., 2006; Brodie *et al*., 2008a; Kucera & Saunders, 2012; Milstein *et al*., 2012; Mols-Mortensen *et al*., 2012). A comparison of markers showed that the mitochondrial encoded *cox*1 performed best at delimiting species while the other markers were more useful for phylogenetic analyses (Kucera & Saunders, 2012; Ramírez *et al*., 2014; Guillemin *et al*., 2016). At present, some drawbacks of using the *cox*1 gene as a routine species-level marker include the deficient database currently available for the bladed Bangiales, introns that hamper amplification, and the potential inability to detect species using this gene due to hybridization or introgression. Introns can increase the size of a targeted amplicon beyond the limits of successful amplification. For this to be resolved, newly designed primers are required that sit upstream of the intron insertion point and therefore amplify a smaller amplicon. Introns are

particularly prevalent in *Pyropia* species, but have also been recorded in other bladed Bangiales (Wang *et al*., 2013; Hughey, 2016). Regarding recently diverging groups, another concern is that two species may share the same *cox*1 gene because of introgression or hybridization, such as *P. umbilicalis* Kützing and *P. linearis* Greville (Mols-Mortensen *et al*., 2012).

Three DNA-based species delimitation methods, the Automatic Barcode Gap Discovery (ABGD), General Mixed Yule Coalescent (GYMC) and Poisson Tree Processes (PTP) have been widely applied recently across a diverse range of organismal groups and are also increasingly used in algal studies (Leliaert *et al*., 2009; Payo *et al*., 2013; Vieira *et al*., 2014; Guillemin *et al*., 2016; Jesus *et al*., 2016; Machín-Sánchez *et al*., 2016; Montecinos *et al*., 2017a). ABGD uses DNA sequence data to delimit species by calculating the barcode gap from pairwise distances among samples (Puillandre *et al*., 2012). GMYC estimates species boundaries by calculating the shift from inter-specific to intraspecific branching rates in a phylogeny by fitting a general mixed Yule-coalescent (GMYC) model on an ultrametric gene tree (Pons *et al*., 2006). PTP estimates species boundaries by modelling the speciation rate directly from the number of substitutions in a phylogeny (Zhang *et al*., 2013). More recently, these DNA-based species methods were applied for the first time to bladed Bangiales, which often lack apparent morphological characters for identification. The study revealed extensive species diversity and endemicity in Chile (Guillemin *et al*., 2016). DNA-based species delimitation using unlinked loci therefore appears promising in resolving the taxonomy of morphologically plastic or cryptic groups such as the Bangiales.

Three bangialean genera occur along the South African coast: the filamentous *Bangia* sensu lato (used hereafter) and the bladed *Porphyra* and *Pyropia*. *Porphyra* occurs from Port St. Johns on the east coast to Port Nolloth on the west coast, spanning a distribution range of ~2000 km of coastline (Isaac, 1957; Graves, 1969; Stegenga *et al*., 1997; Jones *et al*., 2004). Species of *Pyropia* (originally described as *Porphyra* spp.) are only known to occur along the south-west and west coast (Stegenga *et al*., 1997; Jones *et al*., 2004), and *Bangia* has only rarely been observed and collected along the south-west and west coast of South Africa (Stegenga *et al*., 1997; John Bolton personal observation 2016).

Bangiales were first reported from the South African coast by Kützing (1843), who recognized two species of *Porphyra*, a reniform to cordate form (here-after termed 'rosette') and a linear to lanceolate form (henceforth termed 'lanceolate'), both found on the west coast. The rosette form was named *P. capensis* and the lanceolate form, *P. augustinae* Kützing nom. illeg. (see Griffin *et al*. (1999a) for further information regarding the legitimacy of names). The latter species was later synonymized by J. Agardh (1883) and the name *P. capensis* was conserved. Thereafter, two additional species based on European names, *Porphyra vulgaris* C. Agardh nom. illeg. and *Porphyra lacinata* var. *capensis* (Kützing) Grunow, were recorded in South Africa (Delf & Michell, 1921). However, reviews by Isaac

(1957) and Graves (1969) agreed with Agardh and expressed the opinion that only one morphologically variable species, *Porphyra capensis*, occurred in South Africa. However, since then, one new species was described but not named, *Porphyra* sp. indet. (Stegenga *et al*., 1997), and two new *Porphyra* (now *Pyropia*; Sutherland *et al*., 2011) species were described and named, *Py*. *saldanhae* (Stegenga *et al*., 1997) and *Py*. *aeodis* (Griffin *et al*., 1999a). Additionally, two widely distributed *Porphyra* (now *Pyropia*) species, *Pyropia gardneri* and *Py*. *suborbiculata* (as *P*. *carolinensis*) and a cosmopolitan *Bangia* species, *Bangia* cf. *fuscopurpurea* (as *B*. *atropurpurea*) were recorded from South Africa (Stegenga *et al*., 1997; Griffin *et al*., 1999a; Sutherland *et al*., 2011).

Molecular-aided biodiversity studies on the Bangiales in South Africa are largely lacking and to date only a preliminary biodiversity assessment of the bladed Bangiales has been conducted. The study suggested high phylogenetic diversity in the bladed genera (Jones *et al*., 2004; Sutherland *et al*., 2011). The aim of the present study was to further explore the biodiversity of the Bangiales following Jones *et al*. (2004) based on a more extensive collection throughout the known distribution range of these algae along the South African coast.

Because it is well known that data from unlinked loci can provide more reliable estimates of species boundaries (Knowles & Carstens, 2007; Leliaert *et al*., 2014), this study is based on two molecular markers: *cox*1 and *rbc*L, and supplemented with information from a third marker, the nSSU gene. The *cox*1 and *rbc*L genes were sequenced and different algorithmic methods for DNA-based species delimitation (ABGD, GMYC, PTP) applied. Results from these analyses were used to first define initial species hypotheses. Sequences for the nSSU gene were obtained from GenBank and used to generate a multigene phylogeny. Additionally, gross morphological variation and distribution ranges of species were assessed. The species delimited in this study based on DNA-sequence data have to be regarded as hypotheses that should be further tested in future studies using detailed morphological, anatomical, eco-physiological and distributional data.

2.2. MATERIALS AND METHODS

2.2.1. Taxon sampling

Collection sites were selected across the known South African distribution range where Bangiales were found, between East London (33°27’.12’’S, 27°51’.16.52’’E) and Port Nolloth (29°14’.29.4’’S, 16°54’.1.44’’E), including several sites where bladed Bangiales were abundant, particularly on the Cape Peninsula and south-west coast of South Africa (Fig. 1). A survey of Bangiales beyond its known distribution range in South Africa revealed no new records. Specimens were collected during 2014–2016 (Supplementary table S1).

For the purposes of this study, sites from a point east of Suiderstrand to East London were denoted as the south coast, sites between and including Suiderstrand to the Cape Peninsula were denoted as the south-west coast, and sites north of and including the Cape Peninsula were denoted as west coast sites (sensu Stegenga *et al*., 1997). Additional material from samples used in Jones *et al*. (2004) was acquired and amplified for the *cox*1 gene. However, with the exception of three *Pyropia* amplicons that were long enough for comparisons, most of these sequences were too small (~200–300 bp) and were not included in our analyses.

As many different blade forms as possible were collected from various shore positions and from different substrata from 35 sites. Specimens were pressed and preserved as herbarium vouchers, a section from each specimen was removed for DNA analysis and stored in silica gel, and an additional portion preserved in 5% formalin/seawater for anatomical examination. Selected herbarium specimens are deposited in the Bolus Herbarium (BOL) at the University of Cape Town (UCT), South Africa, and all others at the Seaweed Research Unit, Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa.

2.2.2. DNA isolation, PCR-amplification and sequencing

Genomic DNA was extracted using a modified protocol for the DNeasy® Blood and Tissue or Plant Tissue kits (Qiagen Inc.). Approximately 10–20 mg dried algal material was homogenized in liquid nitrogen using a micropestle in 200 µl microcentrifuge tubes. An initial incubation at 56°C for 45 min followed by 80°C for an additional 15 min ensured higher DNA yields. The quality and quantity of DNA was determined using a Nano-Spec® spectro-photometer. DNA concentrations > 20 µg ml^{-1} were diluted 1:10 using distilled water and concentrations lower than 20 µg ml^{-1} were diluted 1:2.

Two partial gene regions were targeted for PCR-amplification, (1) The plastid, *rbc*L and (2) The mitochondrial, *cox*1 genes using published, adapted or newly designed primers (Broom *et al*., 2010; Saunders & Moore, 2013; Supplementary table S2). New primers were designed for two known South African species, *Pyropia saldanhae* and *Py*. *aeodis* and a few *Porphyra* samples that were presumed to contain introns. Primers designed for *Porphyra* specimens were based on an existing *cox*1 dataset. For *Pyropia*, primers were designed based on the species' closest relative (Sutherland *et al*., 2011; Kucera & Saunders, 2012; Lindstrom & Hughey, 2016) because introns were present in all *Pyropia* specimens (Supplementary table S2).

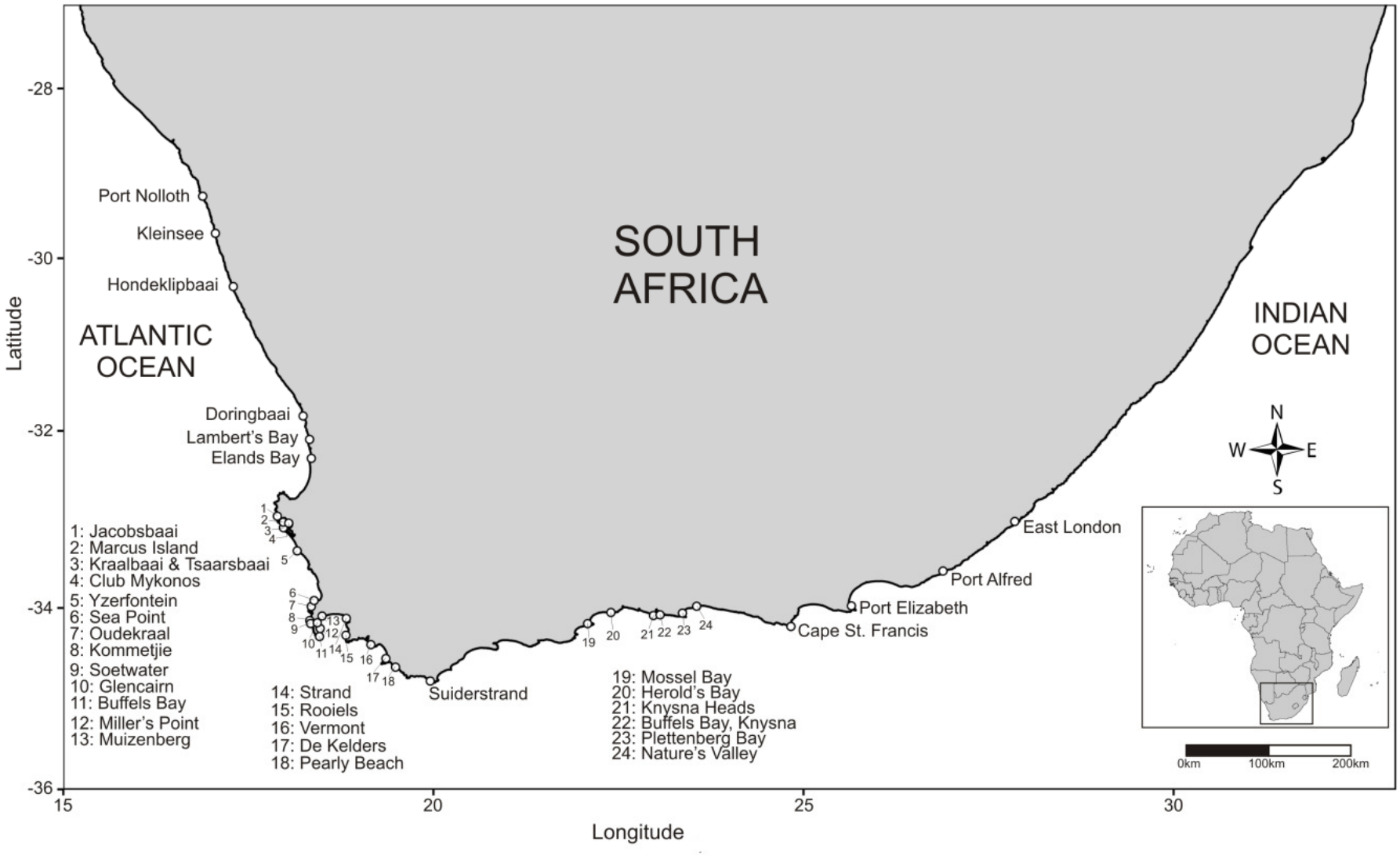

Fig. 2.1. Collection sites along the entire distribution range of the Bangiales in South Africa.

PCR-reactions for the *rbc*L gene contained a final volume of 25 µl, and the concentration of each component was as follows: 1× PCR buffer, 0.2 mM dNTP of each nucleotide, 1 mM $MgCl_2$, 1.25 mM primers, 1.25 u Taq, 1 ug μl^{-1} BSA, 10–30 ng DNA, the volume was made up to the total by adding PCR-grade water (Qiagen Inc.). PCR-reactions were run on an Applied Biosystems Veritit 96-well thermocycler (Life Technologies, USA) or a Biometra Product Line, Professional Thermocycler (Analytik Jena, Germany). PCR thermo-cycling parameters for the *rbc*L gene followed those of Broom *et al*. (2010) with the exception of the annealing temperature which was set at 50°C. PCR-reactions for the *cox*1 gene were the same as above without additional $MgCl_2$. The optimal temperature profile for the *cox*1 gene used a touchdown PCR protocol, an initial denaturation at 94°C for 5 min, 5 cycles of 94°C for 1 min, annealing at 45°C for 1 min 30 s and an extension at 72°C for 1 min 30 s followed by 94°C for 1 min, annealing at 50°C for 1 min 30 s, an extension at 72°C for 1 min 30 s, and a final extension step at 72°C for 5 min.

PCR products were cleaned using an enzymatic digestion (ExoCIAP) and sequenced at Macrogen (Macrogen Inc., Seoul, South Korea) or the Central Analytical Facilities (Stellenbosch, South Africa) sequence facilities. Sequences were submitted to GenBank under the accession numbers KX852772–KX853026, KY814926–KY814952 and KY799110– KY799111.

2.2.3. DNA sequence datasets

Three datasets were generated: *rbc*L, *cox*1 and a concatenated dataset including nSSU sequences. In addition to the sequences produced during this study, a representative selection of published *cox*1, *rbc*L and nSSU sequences for the Bangiales was added to the dataset (Supplementary table S5). In general, for individual gene trees (*rbc*L, *cox*1) three sequences per species were used except where less than three samples were available (Supplementary table S3; Supplementary figs S1– 4). Where several different studies submitted sequences for a single species, one per study was included, therefore *n* per taxon varied from 3–8. A global Bangiales phylogeny following Sutherland *et al*. (2011), but based on a three-gene (*cox*1, *rbc*L, & nSSU) concatenated dataset, and supplemented with new species from updated literature was also reconstructed (Supplementary table S5; Fig. 2; Supplementary fig. S5). DNA sequences were aligned for each gene separately using the Clustal W function in BioEdit (Hall, 1999) and concatenated for the global phylogeny.

2.2.4. Phylogenetic analyses

Each genus was analysed separately, as the inter-generic variation was too high: *Pyropia* species were on average 3× more divergent than *Porphyra* species for the *cox*1 gene (~13%) and 2× more divergent for the *rbc*L gene (~5%). When *Porphyra* and *Pyropia* spp. were initially analysed together, most DNA species delimitation methods failed to detect many known *Porphyra* species as distinct.

The best fitting model for evolution under the Akaike Information Criterion (AIC) was selected for each dataset in Jmodeltest v 2.1.10 (Posada, 2008). For the *cox*1 datasets (*Porphyra*: GTR+I and *Pyropia*: TIM1+I+G) and for the *rbc*L datasets (*Porphyra*: TIM1+I+G & *Pyropia*: GTR+I+G) were implemented in the subsequent phylogenetic analyses. Bayesian inference (BI) and Randomized Accelerated Maximum Likelihood (RAxML) (Stamatakis, 2006) analyses were performed using the programs MrBayes v. 3.2.6 (Ronquist & Huelsenbeck, 2003) and RAxML for web servers (Stamatakis *et al*., 2008), respectively.

The MrBayes analyses consisted of two independent runs of 5 million generations thinning every 1000 trees using four chains (two hot & two cold) to ensure sufficient mixing. Tree parameters were sampled every 1000 generations and independent runs were viewed in Tracer v. 1.5 (Rambaut & Drummond, 2014) to assess convergence and to determine an appropriate burn-in value which was set at 25%. Trees were summarized to create consensus trees and calculate posterior probability values. RAxML was run on the web server RAxML Black Box using default parameters and an appropriate evolutionary model according to Jmodeltest. All trees were rooted on their midpoint.

2.2.5. DNA-based species delimitation methods

ABGD analyses were run on the ABGD web server (wwwabi.snv.jussieu.fr/public/abgd) using the default parameters, except the Kimura K80 distance model which was implemented over the more simplified Jukes-Cantor model and the relative gap width (X) varied depending on the dataset (Table 2). Prior to the GMYC and bPTP analyses, sequences were collapsed into unique haplotypes (Supplementary table S4). For the GMYC analysis, an ultrametric tree was constructed in BEAST v. 1.8 (Drummond *et al*., 2012) using an appropriate model as per Jmodeltest and assuming an uncorrelated lognormal relaxed molecular clock under the constant size coalescent model. Fifty million generations were implemented for two independent runs, sampling every 1000 trees. Runs were inspected for convergence using Tracer v. 1.5 and trees were summarized from the MCMC analyses after discarding the first 25% of trees generated. GMYC analyses were run using a single threshold following Fujisawa & Barraclough (2013) using the SPLITS package in R (R Core Team, 2016). Trees constructed with MrBayes were used as the starting tree for bPTP analyses, which is a Bayesian implementation of the PTP method, run on the web server http://www.exelixis-lab.org/software.html. Singletons refer to a single species, but it is important to note that singletons in haplotypic data may represent several specimens (see Supplementary table S4).

Haplotype networks were constructed for both genera for both genes for which haplogroups and mutations were noted. Pairwise genetic distances (*p*-distances) were calculated in MEGA v. 6.0 (Tamura *et al*., 2013) implemented for 1000 pseudoreplicates.

2.3. RESULTS

A total of 283 sequences (203 *cox*1 & 80 *rbc*L) of South African bladed Bangiales were generated. Sequences for the *cox*1 gene were trimmed to 669 bp, except for a few sequences (those presumed to contain introns) that were shorter in length and trimmed to 350 bp. Intron-containing samples were amplified with alternative primers and therefore produced shorter amplicons. Sequences ranged in size from 864–1409 bp for the *rbc*L gene. South African specimens from this study were resolved in two main clades, corresponding to two genera: *Porphyra* (91% of the *cox*1, & 85% of the *rbc*L sequences) and *Pyropia* (9% of the *cox*1 & 15% of the *rbc*L sequences).

Table 2.1. Basic phylogenetic information for all samples used in the present study for the two partial genes.

	Porphyra		***Pyropia***	
	***cox*1**	***rbc*L**	***cox*1**	***rbc*L**
Total number of sequences / South African (SA) sequences	215 / 185	133 / 75	108 / 18	227 / 12
Total number of haplotypes / number of SA haplotypes	74 / 53	98 / 35	91 / 15	206 / 11
Uncorrected *p*-distance (maximum/average) All sequences/	0.14 / 0.04	0.07 / 0.02	0.19 / 0.05	0.11 / 0.04
SA sequences	0.07 / 0.03	0.04 / 0.02	0.14 / 0.06	0.07 / 0.03

2.3.1. Species delimitation

South African taxa were analysed in the context of already described/named or molecularly identified species obtained from the literature. South African samples, together with GenBank sequences, were partitioned into four datasets, one for each genus (*Porphyra* & *Pyropia*) and for each gene (*cox*1 & *rbc*L) (Table 2.1).

ABGD analyses

Twenty ABGD groups were recovered using the *cox*1 gene for *Porphyra*, and South African taxa accounted for half of these (Table 1.2). The ABGD analysis of the *rbc*L dataset delimited groups that were consistent with the six molecularly identified South African *Porphyra* entities according to Jones *et al*. (2004) (ZPP, ZGR, ZBS, ZCE, ZIR, ZDR). An additional molecular entity (ZSM) from the coast of South Africa identified by Sutherland *et al*. (2011) was not supported as a distinct species but

was instead included in an ABGD group with a number of other species of *Porphyra* such as *P. mumfordii*, *P. linearis* and a few undescribed species (Supplementary fig. S2). The ABGD analysis recovered 41 *Pyropia* groups using the *cox*1 gene, and 93 ABGD groups using the *rbc*L gene. Two taxa were supported as distinct using both markers (*Py. aeodis*, *Py. saldanhae*). SW1, 6POR and ZLI were included in a single group using the *rbc*L gene, but the first two were regarded as distinct species using the *cox*1 gene. 1032 was considered distinct from *Py. aeodis* using the *cox*1 gene but included in the *Py. aeodis* group using the *rbc*L gene (Table 2.2).

Table 2.2. ABGD species groups inferred from two partial gene regions for *Porphyra* and *Pyropia*.

	Porphyra		***Pyropia***	
	***cox*1**	***rbc*L**	***cox*1**	***rbc*L**
Number of sequences	216	133	108	227
X (relative gap width)	1.0	0.95	1.0	0.95
Prior maximal distance for initial partition	$p = 0.002$	$p = 0.003$	$p = 0.005$	$p = 0.005$
Number of ABGD groups	20	26	41	93
South African ABGD groups	10	7	4	3

GMYC analyses

For the *cox*1 dataset of *Porphyra* the GMYC model was favoured over the null model which is that all sequences belong to a single species ($p < 0.01$). Collectively, 17 clusters and six singletons were identified. South African samples were resolved into 10 of these clusters and two singletons (Table 2.3). For the *rbc*L dataset of *Porphyra*, the GMYC model was not favoured over the null model ($p = 0.93$); which is reflected by the large confidence interval (95% CI) in the number of Maximum Likelihood (ML) clusters: 1 to 26 species. For the genus *Pyropia*, the GMYC model was favoured over the null model ($p < 0.01$) using the *cox*1 gene. Twenty-six clusters and 20 singletons were identified, of which four clusters and two singletons represented South African taxa (Table 2.3). Two described South African species (*Py. aeodis* & *Py. saldanhae*) were further split into two and three groups, respectively. Similarly, for the *rbc*L gene, the GMYC model was favoured over the null model. A total of 51 clusters and 54 singletons were delineated. South African taxa were resolved into three clusters and two singletons.

Table 2.3. *Porphyra* and *Pyropia* species delimited using *cox*1 and *rbc*L gene regions implemented in GMYC using a single threshold. CI denotes the confidence interval and SA denotes South Africa.

	Porphyra		***Pyropia***	
	***cox*1**	***rbc*L**	***cox*1**	***rbc*L**
Likelihood of null model	538	815	569	1651
Maximum likelihood (ML) of GMYC model	543	815	597	1678
Likelihood ratio	11 **	0.14 n.s.	6 ***	574 ***
Number of ML	17 (15–22)	25 (1–26)	26 (24–27)	51 (51–54)
Clusters (CI)				
SA ML clusters	10	13	4	3
Number of ML entities (CI)	23 (20–30)	54 (1–97)	46 (42–48)	105 (96–115)
SA ML entities	1	5	2	2

bPTP analyses

A total of 22 *Porphyra* clusters were recovered using the *cox*1 gene; South African taxa accounted for seven clusters and two singletons (Table 2.4). Using the *rbc*L gene, 49 clusters were identified of which eight clusters and four singletons represented South African taxa (Table 2.4). For the genus *Pyropia*, using the *cox*1 gene, 44 clusters were recovered and South African taxa accounted for two clusters (*Py*. *saldanhae* & *Py*. *aeodis*) and two singletons (Table 2.4). Using the *rbc*L gene, 111 clusters were delineated, of which three clusters and two singletons consisted of South African specimens.

Table 2.4. Results of the bPTP analyses based on the *cox*1 and *rbc*L trees for *Porphyra* and *Pyropia*. SA denotes South Africa, ML denotes Maximum Likelihood and BI denotes Bayesian Inference.

	Porphyra		***Pyropia***	
	***cox*1**	***rbc*L**	***cox*1**	***rbc*L**
Acceptance rate	0.44	0.47	0.13	0.25
Estimated number of species	11–42	34–69	40–53	99–124
Mean	22	49	44	111
SA ML clusters	7	8	2	3
Singletons	2	4	2	2
SA BI clusters	7	8	2	3
Singletons	2	4	2	2

Final species hypotheses for South African species

The final species delimitation was based on tabulated results of the different species inferred from each of the two loci (Table 2.5). A 50% majority rule, i.e. when two of the three analytical species delimitation methods (ABGD, GMYC & PTP) were in agreement, was used to decide on consensus species hypotheses following Guillemin *et al*. (2016). More specifically, species clades were recognized when it received high clade support in the *cox*1, *rbc*L, and concatenated phylogenies (*cox*1, *rbc*L, nSSU) and were supported by species-level differences in statistical parsimony and genetic distances (Carstens *et al*., 2013). Additional information on morphology and distribution was taken into consideration when resolving species with unclear boundaries or conflicting results.

In total, 10 species (RSAa–RSAj) of South African *Porphyra* and four species of South African *Pyropia* (RSAk–RSAn) were recognized using the *cox*1 gene. Five were substantiated using the *rbc*L gene: RSAc–d, RSAg, RSAi–j and four supported by the nSSU gene: RSAa, RSAb, RSAi, RSAe. An additional species, ZSM was supported by both *rbc*L and nSSU sequence data. Although, *rbc*L clades were congruent with nSSU clades there were a number of inconsistencies between these clades and the other five *cox*1 species hypotheses (Table 2.5). The following species hypotheses equate to described species: RSAm = *Pyropia aeodis* and RSAn = *Pyropia saldanhae*. RSAn* = the divergent *Py*. *saldanhae* clade.

There was generally high consistency among methods using the *cox*1 gene for *Porphyra* except clades RSAa–b were further split in the GMYC analyses (Supplementary fig. S1). In contrast, there was very little congruence between *rbc*L and *cox*1 GMYC clades for South African *Porphyra* (Supplementary figs S1, S2; Table 2.5). However, in general *Porphyra rbc*L clades compared well with at least half the *cox*1 clades (Supplementary figs S1, S2). On the other hand, all *Pyropia* species hypotheses were generally consistent for both genes and in the concatenated phylogeny, except for RSAl which was consistently recovered as a distinct species using the *cox*1 gene for all methods, but was included in the species *Py*. *aeodis* using the *rbc*L gene (Supplementary figs S3, S4).

Fig. 2.2. A global phylogenetic gene tree based on a concatenated dataset (*cox*1, *rbc*L and nSSU genes) for the Bangiales. South African taxa comprised two genera, highlighted in two subtrees. Support values are indicated at nodes and South African taxa are labelled in red.

Table 2.5. Comparisons of methods and markers and final species delimitation .'*' denotes congruence, L= lumped into a single species, S = Split into multiple species, WC = West coast and SWC = Southwest coast.

FINAL SPECIES DELIMITATION											
Species hypotheses	**Entities (Jones *et al*., 2004)**	**ABGD *cox*1**	**GMYC *cox*1**	**PTP *cox*1**	**ABGD *rbc*L**	**GMYC *rbc*L**	**PTP *rbc*L**	**Concatenated Tree**	**Consensus**	**Distribution**	**Morphology**
***Porphyra* RSAaa**	New	*	L	*	*	*	*	*	*	WC	Rosette
***Porphyra* RSAab**	ZGR/ZBS	L	*	L	L	*	*	*	*	SWC	Lanceolate
***Porphyra* RSAac**	ZGR/ZBS	L	L	L	L	*	*	*	?	WC	Rosette
***Porphyra* RSAba**	ZCE	L	L	L	L	*	*	*	?	SWC	Rosette and Lanceolate
***Porphyra* RSAb**	ZDR	*	S	*	*	S	*	*	*	WC & SWC	Rosette
***Porphyra* RSAc**	New	*	*	L	L	*	*	*	*	SWC	Rosette and Lanceolate
***Porphyra* RSAd**	New	*	*	*	L	*	*	*	*	SWC	Rosette
***Porphyra* RSAe**	ZIR	*	*	*	L	*	*	*	*	WC	Lanceolate
Porphyra	ZIR	*	*	*	L	L	L	*	*	WC	Lanceolate

RSAf											
Porphyra	New	*	*	*	L	*	*	*	*	WC	Lanceolate
RSAg											
Porphyra	ZIR	*	*	*	L	L	L	*	*	WC	Lanceolate
RSAh											
Porphyra	ZPP	*	*	*	L	*	*	*	*	SC & SWC	Rosette
RSAi											
Porphyra	New	*	*	*	L	*	*	*	*	SC	Rosette
RSAj											
Pyropia	ZLI	*	*	*	*	*	*	*	*	WC	Lanceolate to
RSAk											Orbicular
Pyropia	New	*	*	*	L	L	L	*	*	WC	N/A
RSAl											
Pyropia	ZAE=*Py.*	*	*	*	*	*	*	*	*	WC	Cordiform
RSAm	*aeodis*										
Pyropia	ZEK=*Py.*	*	S	*	*	S	*	*	*	WC & SWC	Lanceolate
RSAn	*saldanhe*										

2.3.2. Distribution of species of the bladed Bangiales along the South African coast

RSAi was the only strictly south coast species, containing specimens collected from Port Alfred to Mossel Bay. RSAj represented the other south/south-west coast species and included one specimen collected from De Kelders, ~1000 km west of the remaining eight East London specimens included in this cluster. Both species (RSAi & RSAj) did not overlap in distribution with RSAa–h. All other species hypotheses (RSAa–h) occurred sympatrically, mostly on the west and south-west coast of South Africa, with the exception of five specimens (*Porphyra* sp. CSF 2, KH1, PBB 3, 4, 6) which were collected on the south coast but were included in one of the west coast species.

2.3.3. Morphological variation in Porphyra species

Porphyra species predominantly conformed to one of two morphological forms previously described, rosette or lanceolate (Fig. 2.3). However, in some cases specimens with a lanceolate form were included in an otherwise predominantly rosette species or vice versa. In some species there was an even split between the number of rosette and lanceolate specimens.

Fig. 2.3. Morphological variation in *Porphyra* species along the South African coast. Scale bars represent 25 mm.

2.3.5. Global comparison

Using the *cox*1 gene for *Porphyra*, species groupings were largely consistent with known species using the ABGD, GMYC or PTP methods (Supplementary fig. S2). *Porphyra umbilicalis* and *P. linearis* for the ABGD, GMYC and PTP analyses were recognized as a single species for the *cox*1 gene and all other species groupings were sustained (Supplementary fig. S2). For the *rbc*L gene, results were also largely consistent for known species groupings with some exceptions (Supplementary fig. S3).

For the genus *Pyropia*, when using the *cox*1 gene most known species groupings were sustained (Supplementary fig. S4). For the *rbc*L gene, groupings were consistent for some species, grouped into a single species for others and split into multiple species for some others and a number of mislabelled taxa were evident. For example, *Py. lanceolata* (Setchell & Hus) S.C. Lindstrom was found to appear in more than one species group/clade indicating that the name has been misapplied (Supplementary fig. S5). *Pyropia ishigecola* (Miura) N. Kikuchi and M. Miyata, and *Py. suborbiculata* were considered a single species using the ABGD and PTP methods. These species were retained as mostly separate entities in the GMYC analysis; although the *Py. ishigecola* cluster included a sequence labelled *Py. suborbiculata*.

Genetic distance comparison

Genetic distance matrices were created for each gene (*cox*1 & *rbc*L) and for each genus after checking that names on GenBank were applied correctly at the generic level. A global comparison including known *Porphyra* and *Pyropia* species from the literature was obtained from GenBank and used to calculate intraspecific genetic distances for each genus respectively (Table 2.6). For both genera and for both genes, intraspecific genetic distances of South African species were within range of published distances (Table 2.6). Similarly mutational steps, in statistical parsimony, between South African taxa compared well with differences found in known species.

Table 2.6. Mutational steps calculated from statistical parsimony (SP) and genetic distance (GD) comparisons showing where South Africa taxa fall in the range of species from around the world, based on the *cox*1 and *rbc*L trees for *Porphyra* and *Pyropia*.

	Statistical parsimony			
	Porphyra		***Pyropia***	
	*cox*1	*rbc*L	*cox*1	*rbc*L
Global mutational steps in SP (range)	4–8	4–13	3–9	1–6
	Genetic distances			
	Porphyra		***Pyropia***	
	*cox*1	*rbc*L	*cox*1	*rbc*L
% GD range (average)	1–15 (4)	1–2 (2)	4–21 (13)	1–2 (6)
South African taxa	3–4	1–2	11–14	5–7

2.4. DISCUSSION

Species diversity in the bladed Bangiales in South Africa was studied using different methods of DNA-based species delimitation, and this was interpreted in the context of what is known from other *Porphyra* and *Pyropia* species. Extensive species diversity and endemicity was found along this coastline. Intraspecific genetic distances in South African bladed Bangiales were within the range found in currently defined species based on molecular data (Sutherland *et al*., 2011; Guillemin *et al*., 2016).

Differences in interspecific genetic distances suggest *Porphyra* is a younger clade with more recently radiating species than *Pyropia*. This may explain the higher consistency in analytical species delimitation methods and congruence in markers for South African *Pyropia* species in comparison to *Porphyra* species.

Species boundaries in the bladed Bangiales from around the globe were largely confirmed in this study, although some species displayed high genetic diversity and may consist of multiple species, as has been found in other species groups (Lindstrom & Cole, 1992c; López-Vivas *et al*., 2015; Lindstrom *et al*., 2015a). In contrast, in some other species, even though morphological and/or ecological species criteria were fulfilled, e.g. *P. umbilicalis* and *P. linearis* or *Py. ishigecola* and *Py. suborbiculata*, genetic diversity among these species pairs was extremely low. These results may reflect hybridization or introgression in these species (Mols-Mortensen *et al*., 2012). Misapplied names, either taxa that were misidentified or mislabelled, was another concern when estimating taxonomic diversity for global comparisons.

2.4.1. Comparison of molecular markers and species delimitation methods

Recent studies have demonstrated the value of the *cox*1 gene for delimiting species in the Bangiales as it outperforms other gene markers for this purpose, such as *rbc*L and nSSU (Robba *et al*., 2006; Kucera & Saunders, 2012; Milstein *et al*., 2012). Although the present study generated information for only two markers, information from a third marker was available from a previous study (Jones *et al*., 2004) and this allowed for a comparison of genes. Clearer barcoding gaps were obtained using the *cox*1 gene compared with the *rbc*L and nSSU genes and therefore, *cox*1 was the most effective at delimiting species of South African *Porphyra* and *Pyropia*.

Many recent efforts have focused on adding to the deficient *cox*1 database for the Bangiales (Brodie *et al*., 2008a; Kucera & Saunders, 2012; Mols-Mortensen *et al*., 2012, 2014; Vergés *et al*., 2013b; Sánchez *et al*., 2014, 2015; Milstein *et al*., 2015; Xie *et al*., 2015; Guillemin *et al*., 2016). However, introns remain a problem and in the present study two *Pyropia* species and one *Porphyra* species were

presumed to contain introns in the *cox*1 region. This required designing new primers for intron-containing species which successfully amplified the *cox*1 gene, but with shorter sequence lengths; nevertheless, these sequences were adequate for comparison.

For the genus *Porphyra*, South African specimens were generally included in the same monophyletic species group using nSSU, *rbc*L or *cox*1, but there was some discordance in gene trees for a few specimens. Phylogenetic relationships between South African *Porphyra* species also varied depending on the marker. This may be a result of recent diversification, incomplete lineage sorting, hybridization or introgression (Mols-Mortensen *et al*., 2012; Leliaert *et al*., 2014).

The GMYC method is known to be influenced by completeness of taxon sampling, variability in effective population sizes and the ratio of the effective population size to divergence time, as well as occurrences of rare species (Fujisawa & Barraclough, 2013; Ahrens *et al*., 2016). The method may also fail to resolve recently diverging taxa in some cases (Hudson & Coyne, 2002; Lohse, 2009; Fujisawa & Barraclough, 2013; Talavera *et al*., 2013), or conversely, excessively split species (Miralles & Vences, 2013; Ahrens *et al*., 2016). The excessive splitting in some South African species could, therefore, be accounted for by any of the above mentioned variables.

More specifically, for the genus *Porphyra*, results for GMYC using the *rbc*L gene were not statistically significant and consisted of a large range (1–26 species). A similar trend was observed for other bladed Bangiales studies as well as for other seaweeds (Guillemin *et al*., 2016; Jesus *et al*., 2016). Similarly, ABGD groups tended to sort known species into a single species group. Taken together, these results may reflect the absence of a sufficient barcoding gap in this gene, which essentially reduces the taxonomic resolution of species groupings (Meyer & Paulay, 2005; Meier *et al*., 2008; Kucera & Saunders, 2012). Results from the PTP analysis best reflected species boundaries for *Porphyra* for the *rbc*L gene compared with the other delimiting methods.

On the other hand, analytical methods were congruent using the *cox*1 gene, except for the GMYC results for two *Porphyra* clades and *Pyropia saldanhae* that were further split despite results being statistically significant. These clades consisted of specimens collected from geographically distant sites and the method may be interpreting some level of population structure (Sukumaran & Knowles, 2017). One other consideration is unresolved nodes that may represent real anomalies or methodological artefacts that affect both GMYC and PTP results (Tang *et al*., 2014). In our dataset this is particularly relevant to the aforementioned *cox*1 clades (see for example *Py. saldanhae*).

2.4.2. **Porphyra** *species – identifying the elusive* **P. capensis**

In his initial description, Kützing (1843) referred to *Porphyra capensis* as being rosette in form and the type locality was listed as *Cap* which is regarded as *Caput bonae spei* (South Africa). However, in the 1800s this referred to anywhere between modern day Durban and Cape Town. Hypothetically, even if *P. capensis* was considered to be a typical west coast species, it leaves three or four (RSA a–d) possible species that fit the original description. These rosette species typical of the west coast are consistent with entity ZDR (Jones *et al*., 2004) and a sequence labelled '*P. capensis*' AY766361 on GenBank (Milstein & Oliveira, 2005).

Similarly, the identity of the lanceolate form described as *P. augustinae* nom. illeg. (Kützing, 1843) cannot be confirmed at this time, as the description could refer to any one of the lanceolate west coast *Porphyra* spp. or *Pyropia* spp. found in this study. These genera are morphologically similar to one another and can be distinguished largely on reproductive anatomy and to a lesser extent on ecology: South African *Pyropia* spp. are monoecious and found in the subtidal fringe, only occasionally co-occurring with *Porphyra* spp. (personal observation). The original taxonomic sketches by Kützing (1843) provide no information on spore type or arrangement, or details of the ecology or distribution. Nevertheless, one of the lanceolate species, RSAe–h, is confirmed as consistent with the taxon ZIR (Jones *et al*., 2004). In addition, RSAi recognized in this study is consistent with entity ZPP in Jones *et al*. (2004).

Most other *rbc*L entities, i.e. ZGR, ZBS and ZCE from Jones *et al*. (2004) were either sorted into multiple species or grouped into a single species (RSAa–j), or remained unresolved. An example is the *rbc*L clade ZCE nested in the RSAb clade using the *cox*1 gene.

ZSM (*Porphyra*), a specimen previously collected along the South African coast (Sutherland *et al*., 2011) was not found during this study but was included in the *rbc*L DNA-based species delimitation analyses. The species was shown to be distinct based on the consensus majority rule and will be included in our final species inventory. Taken together, the name *P. capensis*, therefore, cannot be tied to a single species and at present refers to a species complex until the type specimen is sequenced.

2.4.3. Species boundaries confirmed for two endemic **Pyropia** *species*

Two endemic '*Porphyra*' species have been described from among the elusive '*P. capensis*', and were later transferred into the resurrected genus *Pyropia* (Sutherland *et al*., 2011): *Py. aeodis* (Griffin *et al*., 1999a) and *Py. saldanhae* (Stegenga *et al*., 1997). In the present study the boundaries of both species were confirmed and one new *Pyropia* species as well as a divergent lineage within *Py. saldanhae* has been recognized. The novel species, RSAk shares an almost identical *rbc*L

sequence (a single base pair change) with the entity ZLI from a previous study (Jones *et al*., 2004). All analytical DNA-based species delimitations in the present study identified entity ZLI (Jones *et al*., 2004) as being conspecific with RSAk (this study). However, this was not reflected in the multigene phylogeny and may be due to the uneven number of gene regions compared between species (ZLI (*rbc*L & nSSU), RSAk (*rbc*L & *cox*1) and the closely related 6POR (*rbc*L & *cox*1).

A divergent lineage in *Py. saldanhae*, a species that occurs at Rooiels on the eastern shore of False Bay (Fig. 2.1), was found beyond the known distribution range of this species. Previously documented from the Cape Peninsula to Hondeklipbaai (Stegenga *et al*., 1997), the divergent lineage appears morphologically distinct, albeit subtly and will require further morphological and anatomical analyses. Although this lineage was genetically distinct, it was insufficiently so to be considered a distinct species, and as such was consistently recognized as belonging to *Py. saldanhae* using tree-based and non-tree-based species delimitation approaches.

A divergent lineage in *Py. aeodis*, RSAl, acquired from an earlier study along the South African coast (Jones *et al*., 2004) and recognized here as a new entity is represented by only a single specimen which was not available for morphological analysis. Furthermore, the length of the *cox*1 sequence for this sample was significantly shorter than other *Py. aeodis* specimens. For the *rbc*L gene, where a more complete sequence was obtained, this taxon was identified as *Py. aeodis*. Therefore, despite all analytical species delimitation methods and genetic distances based on the *cox*1 gene suggesting this may be a new species, we have chosen not to consider it as such until more information is obtained.

The genus *Pyropia* appears to have relatively fewer species and is much less abundant year-round than *Porphyra* in South Africa, so only relatively few *Pyropia* specimens were sampled. Intraspecific divergence in South African *Pyropia* species was generally high and it is possible that given a larger dataset, more genetic structure and more species may emerge within this genus.

2.4.4. High diversity and regional endemism hidden under common or misapplied names

For many decades the name *Porphyra capensis* Kützing (1843) was used as an umbrella species to describe what we now know to be two divergent genera (*Porphyra* and *Pyropia*) each consisting of several endemic species (Stegenga *et al*., 1997; Griffin *et al*., 1999a; Sutherland *et al*., 2011; this study). Although these genera are morphologically similar, they are markedly genetically distant (this study; Sutherland *et al*., 2011). Even if the name *P. capensis* was restricted to include only *Porphyra* species according to the scheme of Sutherland *et al*. (2011), it still conceals extensive species diversity (10 species). These findings are contrary to earlier reviews by Isaac (1957) and Graves (1969) that considered South African bladed Bangiales belonged to a single species.

Similar trends of high diversity and endemism have also been reported for other regions. For example, the name *Porphyra columbina* Montagne (now *Pyropia columbina* (Montagne) W.A. Nelson) and *P. umbilicalis* have been widely applied to species in New Zealand and Chile, and concealed several endemic and new species along both these coastlines (Broom *et al*., 1999; Nelson *et al*., 2001, 2006; Brodie *et al*., 2007, 2008a; Nelson, 2013; Nelson & D'Archino, 2014; Ramírez *et al*., 2014; Guillemin *et al*., 2016). Widely applied names in North Atlantic bladed Bangiales were also found to conceal cryptic taxonomic diversity (Kucera & Saunders, 2012; Mols-Mortensen *et al*., 2012, 2014).

Misapplied names and misleading distribution ranges

All South African bladed Bangiales identified molecularly in the current study display regional endemism based on the present sampling scheme. However, critical comparisons are needed from subantarctic regions (Gough Island, Tristan da Cunha and Marion Island, where *P. capensis* has been recorded), and from Namibia and southern Angola, where *P. capensis*, *Py. saldanhae* and *Py. aeodis* have been recorded, based on morphological characters (Papenfuss, 1964; Chamberlain, 1965; Silva *et al*., 1996; Anderson *et al*., 2012; John Bolton & Robert Anderson personal observations 2016). Thus, there is a great need for taxonomic clarification of taxa that were previously identified based solely on morphology, particularly with regard to species with a wide range of morphological forms and with wide global distribution ranges (Tronholm *et al*., 2010; Mattio & Payri, 2011; Xie *et al*., 2015).

The widely distributed species, *Pyropia gardneri* which was originally described from California, *Py. suborbiculata* (as *P. carolinensis*) initially described from Japan and *Bangia fuscopurpurea* (as *B. atropurpurea*) which was first described from Germany, were not found in the present study based on DNA sequence data. Furthermore, *Pyropia gardneri* recorded from South Africa (Stegenga *et al*., 1997) morphologically resembles a new endemic South African bladed Bangiales species (RSAk) and requires further study. Therefore, given the difficulty of identifying these species based on morphology (Ramírez *et al*., 2014; Sánchez *et al*., 2014, 2015; Guillemin *et al*., 2016), we suggest that *Py. gardneri* and *Py. suborbiculata* were misidentifications of other species along the South African coast. One other presumed cosmopolitan species, *Bangia* cf. *fuscopurpurea*, was identified based on morphology and recorded along the South African coast. However, no *Bangia* species were found in the present study despite several dedicated seasonal survey trips. Nevertheless, we can conclude with certainty that at least one '*Bangia*' sp. occurs in South Africa but, its identity and endemicity need to be confirmed (Stegenga *et al*., 1997).

The concept of widely distributed macroalgal species has been increasingly challenged in recent times, and many studies reveal regional endemism hidden under widely applied names (Leliaert *et al*., 2009; Payo *et al*., 2013; Vieira *et al*., 2014; Guillemin *et al*., 2016; Jesus *et al*., 2016; Machín-Sánchez

et al., 2016). In the Bangiales, a few common names, generally for well-studied European species such as *P. umbilicalis* (Brodie *et al*., 2008b), have been misapplied to many species from around the world. Similarly, for example, the common European name *P. vulgaris* nom. illeg. has been applied to South African '*Porphyra*' (Delf & Michell, 1921). This is understandable because of a lack of discernible morphological characters in the group, but nevertheless perpetuates the idea of widely distributed bangialean species.

2.4.5. Bangialean species inventory in South Africa

These analyses suggest that 14–16 species of Bangiales occur along the South African coast, three of which have been previously described and named (*Porphyra capensis*, *Pyropia saldanhae* & *Py. aeodis*). The name *Porphyra capensis* cannot be reliably assigned to a single species and instead refers to a complex consisting of 10 species. The *Porphyra* genetic entity ZMS which was not found in the present study, but for which molecular sequences exist (*rbc*L & nSSU) was included in the final species list. In addition to two species of *Pyropia* endemic to southern Africa, a new species of *Pyropia* is identified, RSAk. Therefore, in total 14 species are recognized, all of which can be attached to molecular sequences. The final estimate included two additional species that require verification. These were *Bangia* cf. *fuscopurpurea* and *Pyropia* cf. *suborbiculata*, the identity and generic placements of which, however, need to be determined. The endemic, *Porphyra* sp. indet. (Stegenga *et al*., 1997), has not been found again since its description and it is therefore doubtful that this species represents a distinct entity. All three of these species are currently lacking molecular data. For reasons mentioned above, the widely distributed *Py. gardneri* has been provisionally removed from the South African flora until further research is conducted. Earlier taxonomic circumscriptions that were synonymized with *P. capensis* (*P. augustinae* nom. illeg., *P. vulgaris* nom. illeg. and *P. lacinata* var. *capensis* (Kützing) Grunow) were also excluded from our final inventory.

2.5. CONCLUSION

In conclusion, the present study found extensive diversity, regional endemism and geographic structure in the Bangiales along the South African coast. Phylogenetic diversity was considered in the context of currently accepted species boundaries, using different DNA-based species delimiting methods and a multigene phylogeny. The relative efficacies of these methods were compared and despite some differences, a high level of congruence was found between molecular markers and methods. These results demonstrate the value of applying a statistical framework when defining species boundaries in taxonomically challenging groups such as the Bangiales; allowing for reproducibility while minimizing the inherent subjectivity associated with defining species boundaries. Although several established species boundaries from other regions outside South Africa were affirmed, these analyses suggest that a high level of species diversity is waiting to be discovered.

In particular, the South African coast proved to be a repository for undiscovered species and although the present study was based on an extensive collection throughout the known distribution range of the Bangiales, species are known to occur seasonally and further sampling may result in the recognition of more species from this coastline. In the present study, species were based on molecular information and these species hypotheses need to be further explored using detailed morphological, anatomical and distributional data. These findings provide a good indication of the total number of Bangiales in South Africa and largely contribute toward understanding the biodiversity of the Bangiales on a global scale. Furthermore, this study forms the basis for future research on the evolution, ecology and biology of this hyper-diverse species complex in the Southern Hemisphere. Lastly, future work should focus on identifying commercially important species/strains from South Africa.

CHAPTER 3

A Reappraisal of the Genus *Pyropia* (Bangiales, Rhodophyta) from Southern Africa Based on a Multi-Gene Phylogeny, Morphology and Ecology, Including the Description of *Pyropia meridionalis* sp. nov

3.1. INTRODUCTION

The recently resurrected *Pyropia* J. Agardh is the most speciose and widely distributed genus in the Bangiales (Sutherland *et al*., 2011; Lim *et al*., 2017). Species belonging to the genus are well-known for their commercial cultivation in the multibillion-US dollar nori industry (Yang *et al*., 2017a). Additionally, they are notorious for their complicated taxonomic history, due to their simple but variable thallus morphology, colour and blade thickness.

A large majority of species (*ca*. 75–80%) that were initially assigned to the genus *Porphyra*, were transferred to the genus *Pyropia*, following a taxonomic revision of the Bangiales based on molecular data (Sutherland *et al*., 2011). This together with the use of molecular techniques in species discovery has resulted in a great increase in the number of species of *Pyropia* worldwide (Broom *et al*., 1999, 2004, 2010; Nelson *et al*., 2001, 2006, 2013; Mols-Mortensen *et al*., 2012; Mateo-Cid *et al*., 2012; Nelson & D'Archino, 2014; Ramírez *et al*., 2014; Sánchez *et al*., 2014, 2015; Lindstrom *et al*., 2015a, b; Guillemin *et al*., 2016). More specifically many species have recently been discovered in the Southern Hemisphere, where they tend to be regionally confined (Nelson *et al*., 2001, 2006, 2013; Brodie *et al*., 2007, 2008b; Mateo-Cid *et al*., 2012; Nelson & D'Archino, 2014; Ramírez *et al*., 2014; López-Vivas *et al*., 2015; Guillemin *et al*., 2016; Reddy *et al*., 2018). Despite this, many regions in the Southern Hemisphere as well as subtidal habitats in both the Southern and Northern Hemispheres remain relatively unexplored and may harbour a diversity far exceeding that which is currently known (Broom *et al*., 2010; Sutherland *et al*., 2011; Nelson *et al*., 2014; Koh *et al*., 2016; Reddy *et al*., 2018).

Pyropia saldanhae (formerly *Porphyra saldanhae*) was the first species of *Pyropia* recognized from southern Africa (Stegenga *et al*., 1997). It is endemic to the Benguela Marine Province, occurring along the lower intertidal and sublittoral fringe, on rocky substrata along the west coast of South Africa (Stegenga *et al*., 1997) or epiphytically on *Ahnfeltiopsis vermicularis* (C. Agardh) P.C. Silva & DeCew or *Pachymenia orbitosa* (Suhr) L.K. Russell (formerly *Aeodes orbitosa* (Suhr) Schmitz) along the Namibian coast (Lluch, 2002). The occurrence of *Pyropia saldanhae* in South Africa has been confirmed using molecular sequence data (Jones *et al*., 2004; Sutherland *et al*., 2011; Reddy *et al*., 2018) while distribution records outside this region are based on traditional methods of identification using morpho-anatomical characters (Lluch, 2002).

Along with *Porphyra saldanhae*, Stegenga *et al*. (1997) also described and illustrated an unnamed species (*Porphyra* sp. indet.) and recorded *P. gardneri* and *P. carolinensis* from South Africa. *Porphyra carolinensis* has subsequently been reduced to a synonym of *P. suborbiculata* (Broom *et al*., 2002) and *P. saldanhae*, *P. gardneri* and *P. suborbiculata* have since been assigned to the genus *Pyropia* (Sutherland *et al*., 2011). *Porphyra* sp. indet. and *Pyropia gardneri* differ only slightly based

on morpho-anatomical characteristics (Stegenga *et al*., 1997; Lluch 2002; personal observation) and are both epiphytic on kelp. *Pyropia gardneri* was recorded along the south-west coast and *Porphyra* sp. indet. along the west coast of South Africa. The latter has not been collected since it was first described and is currently represented by a single herbarium specimen in BOL. Additionally, Lluch (2002) identified an unnamed epiphytic species (*Porphyra* sp.) from Namibia with morpho-anatomical features intermediate between *Pyropia gardneri* and *Porphyra* sp. indet. as described by Stegenga *et al*. (1997).

Two years after the description of *Porphyra saldanhae*, an epiphytic species endemic to southern Africa, *Porphyra aeodis*, was described (Griffin *et al*., 1999a), but later transferred to the genus *Pyropia* (Sutherland *et al*., 2011). Griffin *et al*. (1999a) were the first to include molecular data (isozymes), in addition to morphology, anatomy and ecology, in delimiting southern African Bangiales. *Pyropia aeodis* is a summer annual and is epiphytic on *Pachymenia orbitosa* which occurs along the lower intertidal and sublittoral fringe. Although only confirmed molecularly from South Africa, it is reported (based on morphology) as extending into northern Namibia (Griffin *et al*., 1999a). Its distribution thus parallels that of *Py. saldanhae* along the Benguela Marine Province (Stegenga *et al*., 1997; Griffin *et al*., 1999a; Maggie Reddy personal observation 2014–2018).

Simple morphologies with overlapping anatomical and ecological characters make it difficult to distinguish *Py. saldanhae* from *Py. aeodis*. They are co-distributed geographically and along the lower intertidal and sublittoral fringe, and both species have two stellate chloroplasts per cell, and monoecious gametophytes with intermingling spermatial and zygotosporangial sori (Stegenga *et al*., 1997; Griffin *et al*., 1999a). Although slight differences in their reproductive anatomy have been reported, such as *Py. aeodis* having 8–16 tiers of spermatangia and *Py. saldanhae* having only eight tiers of spermatangia (Stegenga *et al*., 1997; Griffin *et al*., 1999a), the number of tiers of spermatia overlaps and may not always be diagnostic. Ecological traits, such as substratum affinity (epilithic vs. epiphytic), seasonality, and ultimately molecular signatures are better at distinguishing these morphologically similar species (Griffin *et al*., 1999a; Jones *et al*., 2004).

A bangialean biodiversity assessment along the South African coast in 2004 by Jones *et al*. identified much higher species diversity than previously recorded. Their study used the nuclear SSU rRNA (nSSU), including the variable v9 region, to assess species diversity. Eleven entities were recognized, and three of these (*P. saldanhae*, *P. aeodis* & the new molecular species *Porphyra* ZLI) have been transferred or identified to belong to the genus *Pyropia* (Sutherland *et al*., 2011). More recently, *Pyropia* ZLI was identified as being conspecific with the molecularly identified species *Pyropia* RSAk during a comprehensive biodiversity assessment of the Bangiales along the South African coast (Reddy *et al*., 2018). The presence of two widely distributed species, *Py. gardneri* and

Py. *suborbiculata*, as well as the endemic *P*. sp. indet could not be confirmed in either study (Jones *et al*., 2004; Reddy *et al*., 2018).

Currently, seven species of *Pyropia* have been documented from the Benguela Marine Province in southern Africa (South Africa & Namibia): *Py*. *saldanhae* and *Py*. *aeodis*, based on morphology and confirmed using molecular sequence data, *Py.* RSAk (= *Py.* ZLI) using only molecular sequence data, as well as *Py*. *gardneri*, *Py*. *suborbiculata*, *P.* sp. indet. (Stegenga *et al*., 1997) and *Porphyra* sp. (Lluch, 2002) based only on morphology. The aim of the present study was to characterize the morphology, ecology and geographical distributions of southern African *Pyropia* species, including the recently DNA-based delimited species in Reddy *et al*. (2018). First, the species previously identified molecularly as RSAk = *Pyropia* ZLI was formally described and anatomical, morphological and ecological information provided for this species. Additionally, information on the description, distribution and ecology of *Py*. *saldanhae* and *Py*. *aeodis* is updated, and photomicrographs of key anatomical features are provided. The identity of two species of uncertain taxonomic status; *Porphyra* sp. indet. (Stegenga *et al*., 1997) and *Porphyra* sp. (Lluch, 2002), as well as two widely distributed species: *Py*. *gardneri* and *Py*. *suborbiculata* recorded from southern Africa were examined using morphological and anatomical features. Lastly, the phylogenetic affinities of southern African *Pyropia* in relation to species from around the world were discussed using additional sequence data.

3.2. MATERIALS AND METHODS

3.2.1. Taxon sampling

New specimens were collected along the south-west and west coasts of South Africa and herbarium specimens from South Africa and Namibia were re-examined (Table 3.1); no fresh material fitting the description of *Py*. *suborbiculata* or *P*. sp. indet. was found. For RSAk, numerous individual bladelets (*ca*. 30) were found growing on a single shell of the kelp stipe limpet, *Cymbula compressa* Linnaeus, from a stipe of *Ecklonia maxima* (Osbeck) Papenfuss, and were anatomically identical and thus presumed to constitute the same species. Voucher specimens are deposited in BOL and the Seaweed Research Unit, Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa. Blades were preserved in a 5% buffered formalin/seawater solution for anatomical assessment. Sections were prepared by hand with a scalpel under a dissecting microscope and were water-mounted on slides. Slides were viewed under a Leica Wild M10 light microscope coupled to an Olympus D50 digital camera. Representative photographs were taken of vegetative and reproductive cells (when present), in cross section and surface view of the thallus. A portion of each blade was stored in silica gel for molecular analysis.

3.2.2. Herbarium collections

Herbarium abbreviations follow Thiers (2018). The collections of four herbaria, BOL (Cape Town, South Africa), GRA (Grahamstown, South Africa), NU (KwaZulu-Natal, South Africa) and GENT (Ghent, Belgium) were consulted, for specimens that fit the morphological description of RSAk and that were associated with kelp, but specimens were only found in BOL. Herbarium records from BOL were also re-examined for all other *Pyropia* species recorded from southern Africa (*Py. saldanhae*, *Py. aeodis*, *Porphyra* sp. indet., *Py. gardneri* & *Py. suborbiculata*) except *Porphyra* sp. (Lluch, 2002). Details from these records and sequence data from a previous study (Jones *et al*., 2004) were added to this study. No description or anatomical features were detailed by Stegenga *et al*. (1997) for *Py. suborbiculata* because it did not occur in their study region (the west coast of South Africa). The morpho-anatomical features of this species from rehydrated material from the herbarium specimen 15251 (BOL) was therefore examined. Anatomical features for all other species are presented in Stegenga *et al*. (1997), Griffin *et al*. (1999a) and Lluch (2002). No specimens from herbarium collections were sequenced because they were believed to be formalin-treated and unsuitable for DNA analyses.

3.2.3. DNA extraction, PCR amplification and sequence acquisition

Small blade fragments (10–20 mg) were homogenized using liquid nitrogen. DNA was isolated using the DNeasy® Blood and Tissue or Plant Tissue kits (Qiagen, Hilden, Germany) following the manufacturer's protocols with slight modification (see Chapter 2). Three gene regions were targeted for amplification: *cox*1, *rbc*L and nSSU genes. Primer choice or design, PCR optimisation and amplification for the first two genes followed the same methods as Chapter 2. The nSSU gene was amplified in two fragments using primers from Jones *et al*. (2004). PCR amplicons were purified and sequenced at Macrogen (Macrogen Inc., Seoul, South Korea) or Inqaba Biotechnical Industries (Pretoria, South Africa).

3.2.4. Phylogenetic analyses

Four DNA datasets were generated (one for each gene) and a concatenated species dataset. Additional sequences of species that share a close phylogenetic relationship with southern African species were acquired from GenBank. Sequences were aligned and edited in BioEdit (Hall, 1999). An evolutionary model that best explained the data was calculated for each gene region in Jmodeltest v 2.1.10 (Posada, 2008) for each dataset.

Bayesian Inference (BI) trees were generated in MrBayes v. 3.2.6 (Ronquist & Huelsenbeck, 2003). Two parallels runs were implemented for 5 million generations each and sampling every 1000 trees,

using incrementally heated chains (two hot & two cold). Runs were assessed for stationarity using Tracer v. 1.5 (Rambaut & Drummond, 2014). Trees from both runs were combined and 25% discarded before constructing a 50% majority rule tree and calculating posterior probability values. Maximum Likelihood (ML) trees were constructed in Randomized Accelerated Maximum Likelihood (RAxML) for web servers (Stamatakis, 2006; Stamatakis *et al*., 2008) using default settings and an appropriate model according to Jmodeltest. Posterior probability and bootstrap values were presented on a single phylogenetic tree. A *cox*1 tree, including related species from around the globe, was constructed to confirm the phylogenetic position of southern African species of *Pyropia*, including new sequences generated in this study. Further, a phylogenetic tree (species tree) was generated based on a concatenated alignment, to infer phylogenetic relationships and affinities of southern Africa taxa. Lastly, pairwise genetic distance comparisons were calculated in MEGA v. 6.0 (Tamura *et al*., 2013).

Table 3.1. Specimen list of species of *Pyropia* along the South African coast including new collections and herbarium specimens examined: (name, date, ref number).

New collections									
Species	**Location**	**Coordinates**	**Substratum**	**Season (Austral)**	**Source and voucher specimen numbers**	**Remarks**	**Accession numbers**		
							***cox*1**	***rbc*L**	**nSSU**
Py. RSAk (= *Py. meridionalis*)	Kleinzee, South Africa	-29.707946, 17.054532	Epizoic on *C. compressa*	Autumn	(Seaweed unit, KZ 8–10, 29/03/2017, D2865)	Collected from 2–3 m deep relative to the low tide level	2083518		
	Hondeklipbaai, South Africa	-30.307152, 17.269093	Epizoic on *C. compressa*	Autumn	(Seaweed unit, HK 9–11, 30/03/2017, D2866–D2868)	Collected from 1.5 m deep relative to the low tide level	2083522		
	Doringbaai, South Africa	-31.821056, 18.239235	Epizoic on *C. compressa*	Autumn	(Seaweed unit, DB 7–9, 31/03/2017, D2870–D2872)	Collected from > 1 m deep relative to the low tide level	2083525		
	Paternoster, South Africa	-32.804466, 17.883083	Epizoic on *C. compressa*	Summer	(Jones *et al*. 2004, Jan–Feb 2001, WELT A23073)	(as *Py*. ZLI1045)		GU165839	AY292635
		-32.804466, 17.883083	Epizoic on *C. compressa*	Spring	(Seaweed unit, PTN 1–3, 18/11/2016, D2738–D2740)	Collected from 2 m relative to the low tide level	2083526		
	Soetwater,	-34.174437,	Epizoic on *C.*	Summer	(Anderson,	Holotype	KY814951	KY814952	

	South Africa	18.335948	*compressa*		SW1–3, 24/02/2015, D2257, BOL201158)	Collected from 1–2 m relative to the low tide level			
	Muizenberg, South Africa	-34.109955, 18.468441	Epizoic on *C. compressa*	Summer	(Reddy, MZ11–12, 05/12/2016, D2741–D2742)	On drift kelp	2083528–9		
Py. saldanhae	Hondeklipbaai, South Africa	-30.306950, 17.269776	Epiphytic on *E. maxima*	Autumn	(Seaweed unit, HK12, 30/03/2017, D2869)		2083621		
	Paternoster, South Africa	-32.804466, 17.883083	Epizoic on mussels	Summer	(Jones *et al*. 2004, Jan–Feb 2001, MATS1032)	As ZEK881 (*Py. saldanhae*)			AY292630
	Jacobsbaai, South Africa	-32.976377, 17.882297	Epilithic, sublittoral fringe	Winter	(Reddy, JC4, 09/08/2014, D1991)		KY814931	KY814943	
		-32.976377, 17.882297	Epizoic on mussels, sublittoral fringe	Winter	(Reddy, JC8, 09/08/2014, 1995)		KY814932	KY814944	
		-32.976377, 17.882297	Epizoic on mussels, sublittoral fringe	Winter	(Reddy, JC9,09/08/2014, 1996)		KY814937		
		-32.976377, 17.882297	Epilithic, sublittoral fringe	Winter	(Reddy, JC11, 08/06/2016, 1998)		KY814936		
		-32.976377, 17.882297	Epiphytic on *E. maxima*	Winter	(Seaweed unit, JC36, 08/06/2016,	Collected from 2 m deep	2083526		

					D2707)	relative to the low tide level		
	Tsaarbaai, South Africa	-33.096213, 17.973956	Epilithic, mid to low intertidal	Winter	(Reddy, TS6, 10/08/2014, D2029)		KY814934	KY814946
		-33.096213, 17.973956	Epiphytic on *P. orbitosa*, sublittoral fringe	Winter	(Reddy, TS20, 10/08/2014, D2043)		KY814933	KY814945
		-33.096213, 17.973956	Epizoic on mussels, sublittoral fringe	Winter	(Reddy, TS22, 10/08/2014, 2045)		KY814942	
	Kommetjie, South Africa	-34.1403, 18.3292	Epiphytic on *E. maxima*	Summer	(Jones *et al.* 2004, Jan–Feb 2001, MATS1058)	KMD3 (1058), as *Py. saldanhae*	KY814935	GU165838
	Rooiels, South Africa	-34.303534, 18.813502	Epizoic on mussels, sublittoral fringe	Winter	(Reddy, RE2, 15/07/2015, D2350)		KY814939	KY814947
		-34.303534, 18.813502	Epizoic, mid to low intertidal	Winter	(Reddy, RE8, 15/07/2015, D2356)		KY814940	KY814948
		-34.303534, 18.813502	Epizoic, mid to low intertidal	Winter	(Reddy, RE9, 15/07/2015, D2357)		KY814941	KY814949
		-34.303534, 18.813502	Epizoic, mid to low intertidal	Winter	(Reddy, RE10, 15/07/2015, D2358)		KY814938	KY814950
	Muizenberg, South Africa	-34.109739, 18.468735	Epiphytic on *E. maxima*	Summer	(Reddy, MZ14, 05/12/2016, D2744)	On drift kelp	2083625	
Py. aeodis	Paternoster, South Africa	-32.804466, 17.883083	Epizoic on mussels, mid to low intertidal	Summer	(Jones *et al.* 2004, Jan–Feb 2001,		KY81492	KY814930

		-32.804466, 17.883083	Epizoic on mussels, mid to low intertidal	Summer	MATS1032) (Jones *et al*. 2004, Jan–Feb 2001, MATS1025)	ZAE953 (*Py. aeodis*)		GU165843	AY292624
	Yzerfontein, South Africa	-33.354290, 18.149521	Epilithic, mid to low intertidal	Winter	(Reddy, YZ10, 17/08/2014, D2088)		KY814926		
	Marcus Island, Langebaan, South Africa	-33.043540, 17.966483	Epilithic, mid to low intertidal	Autumn	(Reddy, MI3, 23/04/2015, D2196)		KY799110	KY814929	
		-33.043540, 17.966483	Epilithic, mid to low intertidal	Autumn	(Reddy, MI12, 23/04/2015, D2205)		KY814926		
	Scarborough, South Africa	-34.199011, 18.371302	Epiphytic on *P. orbitosa*	Autumn	(Reddy, SB3–5, 02/04/2017, D2875–D2877)		2083506		
	Kommetjie, South Africa	-34.1403 18.3292	Epiphytic on *P. orbitosa*, lower eulittoral	Summer	(Anderson, KM9, 07/01/2015, D2156)		KY799111	KY814928	
Herbarium collections									
Py. RSAk (= *Py. meridionalis*)	Kei Mouth, South Africa	-32.683688, 28.382625	Epiphytic on *E. radiata*	Spring	(Stegenga, 23/10/1999, BOL 15257)				
	Lamberts Bay, South Africa	-32.096502, 18.301840	Epizoic on *C. compressa* (as *Patella compressa*),	Autumn	(Stegenga, 8/3/1985, BOL 15289)				
	Glencairn, South Africa	-34.161961, 18.431692	Epiphytic on stipes of *E. maxima*	Spring	(Stegenga, 1/9/1989, BOL 15274)				

	Brandfontein (West of Cape Agulhas), South Africa	-34.7670, 19.8670	Epiphytic on *E. maxima*	Spring	(Bolton and Stegenga, 11/11/1989, BOL 15272),	
		-34.7670, 19.8670	Epiphytic on *E. maxima*	Spring	(Bolton and Stegenga, 11/11/1989, BOL 15273)	
	Melkbosstrand, South Africa	-33.7333, 18.4333	Epiphytic on stipes of *E. maxima*	Winter	(Stegenga, 26/8/1991, BOL 15270)	
	Skaapeiland, Yzerfontein, South Africa	-33.354290, 18.149521	Epiphytic on *Laminaria pallida*	Spring	(Bolton and Stegenga, 26/11/1988, XX)	
		-33.354290, 18.149521	Epiphytic on *L. pallida*	Spring	(Bolton and Stegenga, 26/11/1988, BOL 15267),	
	Morgan Bay, South Africa	-32.710573, 28.340077	Epiphytic on *E. radiata*	Spring	(Stegenga, 24/10/1999, BOL 15252),	(as *Py. gardneri*)
	Port Nolloth, South Africa	-29.241007, 16.857179	Epizoic on *C. compressa* (as *P. compressa*),	Summer	(Simons and Graves, 23/1/1958, XX)	
Py. saldanhae	Olifantsbos, Cape Point, South Africa	-34.258271, 18.381107	Epilithic, low intertidal	Summer	(Bolton and Stegenga, 21/12/1988, BOL 15263)	
	Cape Fria, Namibia	-18.468415, 12.021122	N/A	Autumn	(Engledow, 16/04/1992, BOL 15276)	

	Yzerfontein, South Africa	-33.354290, 18.149521	N/A	Spring	(Stegenga, 26/10/1988, XX)	
		-33.354290, 18.149521	N/A	Spring	(Bolton and Stegenga, 26/10/1988, BOL 15255)	Holotype
	Rocklands, Table Bay, South Africa	-33.9034, 18.4207	Site cleared of *Scutellastra cochlear*	Autumn/Winter	(Joska, 20/04 to18/06 1981, BOL 15372)	
		-33.9034, 18.4207	Site cleared of *S. cochlear*	Autumn	(Joska, 20/03 to 19/04 1981, BOL 15374)	
		-33.9034, 18.4207	Site cleared of *S. cochlear*	Spring	(Joska, 14/10/1981, BOL 15367)	
Py. aeodis	Möwe Bay, Namibia	-19.371483, 12.705231	Epiphytic on *P. orbitosa*	Autumn	(Engledow, 13/04/1992, BOL 15279)	
	Möwe Bay, Namibia	-19.371483, 12.705231	Epiphytic on *P. orbitosa*	Autumn	(Engledow, 13/04/1992, BOL 15280)	
	Agate Bay, Namibia	-26.608528, 15.175471	Epiphytic on *P. orbitosa*	Autumn	(Engledow, 17/03/1992, BOL 15260)	
	Hondeklipbaai, South Africa	-30.306950, 17.269776	Epiphytic on *P. orbitosa*	Summer	(Stegenga, 16/02/1992, BOL 15265)	
	Hondeklipbaai, South Africa	-30.306950, 17.269776	Epiphytic on *P. orbitosa*	Summer	(Stegenga, 19/01/1989, BOL 15262)	Drift
	Kommetjie, South Africa	-34.1403, 18.3292	Epiphytic on *P. orbitosa*, eulittoral	Autumn	(Griffin, 16/05/1995, BOL 150073)	Holotype
	Kommetjie, South Africa	-34.1403, 18.3292	Epiphytic on *P. orbitosa*, eulittoral	Autumn	(Griffin, 16/05/1995, BOL 150074)	Isotype

	Kommetjie, South Africa	-34.1403, 18.3292	Epiphytic on *P. orbitosa*, eulittoral	Autumn	(Griffin, 16/05/1995, BOL 150075)	Isotype
Py. suborbiculata	Cape Infanta, South Africa	-34.422416, 20.856342	Epilithic in rock pool	Spring	Stegenga, 24/09/1984, BOL 15251)	
P. sp. indet.	Melkbosstrand, South Africa	-33.727840, 18.437990	Epiphytic on stipes of *E. maxima*	Summer	(Stegenga, 9/12/1988, BOL 15264)	

*Accession numbers have not been received for all sequences and for those missing accession numbers GenBank submission reference number have been provided.

3.3. RESULTS

3.3.1. A description of the genus **Pyropia** *J. Agardh in South Africa*

Gametophyte thalli bladed, membranous and monostromatic varying in colour from red-brown to deep maroon. Blades lanceolate, cordiform to umbilicate, spatulate or orbicular and ranging in size from a few mm to more than a metre. Gametophyte phase alternating with a filamentous *conchocelis*-phase similar to *Porphyra*. Monoecious habit with a characteristic intermingling of male and female reproductive cells. Female reproductive cells with trichogynes on opposite ends. Gametophytes found growing on a range of substrata, epizoic, epiphytic or epilithic and generally occurring in the lower eulittoral or sublittoral.

3.3.2. Taxonomic treatment

A total of 70 specimens (26 herbarium specimens & 44 new collections) were analysed in the present study. Three species of *Pyropia* from the southern African coast: *Py. saldanhae*, *Py. aeodis* and *Py. meridionalis* sp. nov. (described below) are validated in the present study, and a further species that is morphologically similar to *Py. suborbiculata* is detailed. Specimens of '*Py.gardneri*' reported from South Africa were anatomically and molecularly identical to *Py. meridionalis*. The original collection site of *P.* sp. indet. was thoroughly searched but no further specimens found. Nevertheless it was evident that a number of morphological and anatomical features of this species, as illustrated by Stegenga *et al*. (1997) and *Porphyra* sp. as illustrated by Lluch (2002), overlap with that of *Py. meridionalis*. Illustrations of *Py.gardneri*, *Py.* sp. indet and *Porphyra* sp. can be found in Stegenga *et al*. (1997) and Lluch (2002), and are not presented here. However, details of these species are available in Table 3.2 for comparative purposes.

The separate phylogenetic placement (Fig. 3.5) and distinct morphological, anatomical and ecological features warrant the description of a new southern African species of *Pyropia*.

Pyropia meridionalis sp. nov. **M.M. Reddy, R.J. Anderson & J.J. Bolton**

Diagnosis: Epibiont on kelp (epiphytic on kelp or epizoic on kelp limpets) with extremely thin, ribbon-like or spathulate thalli with somewhat undulate margins and tapering toward a distinct stipitate base. Thalli single-bladed, 25–50 (–150) mm long and 10–15 mm wide. Thalli lanceolate to obovate or spathulate, spathulate thalli with a broad, rounded apex and tapering to an extremely narrow stipe-like base; margins entire. Apex generally broader than base and the whole thallus 2–4 times longer than broad. Margins somewhat undulate basally continuing up to at least ¾ of the length of the thallus, the remaining length comprising the apex with smooth and entire margins. Fertile tissue

in two distinct bands, along either side of the thallus margin, near the apex in smaller plants and more basal in larger plants. Fertile tissue pale yellow (male) or pink (female). Colour of fresh thalli ranging from pale copper, pale pink, pale ruby to pale tawny, sometimes more greenish at the base; when dried becoming more pale pink or purple with the base remaining darker and the apex lighter. Blades monostromatic, 20–40 (–60) µm thick, thinner in vegetative regions or smaller thalli and thicker in reproductive regions or larger thalli. In cross section, non-reproductive cells 12.5–13 µm long x 10–11 µm wide and squarish to rectangular in shape, only slightly longer than wide in rectangular cells; cells each with a single stellate chloroplast. In surface view, non-reproductive cells compact and arranged in longitudinal rows, often in pairs. Some cells in surface view appear characteristically rectangular to square; cells near base of thallus longer (elongated protrusions at one or more corners of the cell). Reproductive cells in cross section larger than vegetative cells (*ca*. 20 µm long by 15 µm). Monoecious, with spermatia generally smaller than zygotosporangia, bright yellow to golden and lanceolate to fusiform. Spermatia present in pairs, in 8–16 tiers. Female reproductive cells often in pairs, each with distinct bipolar trichogynes with a single cell dividing in the latitudinal plane. Zygotosporangia larger than spermatia, red to maroon and elliptical to round, present in pairs, in 2–4 tiers. Spermatia distinguished from zygotosporangia by appearance (see above). Reproductive cells in surface view arranged in sets of 4, more compact than non-reproductive cells. Reproductive cells commonly observed toward the outer edges of the thallus margin (distinct bands) and zygotosporangia toward the thallus centre, sometimes in isolated islands amongst spermatia.

Holotype: BOL201158 (Soetwater, Anderson, 24/02/2015, D2257).

Epitypes: BOL201159 (Doringbaai, Seaweed unit, 31/03/2017, D2870); BOL201160 (Hondeklipbaai, Seaweed unit, 30/03/2017, D2866).

Representatives DNA sequences: KY814951 (*cox*1), KY814952 (*rbc*L) and AY292635 (nSSU).

Type locality: Soetwater, Cape Peninsula, South Africa.

Etymology: This species is named for its distribution along the coastline of southern Africa, (*meridionalis* in Latin translates to southern).

Distribution: South Africa and Namibia (Benguela & Agulhas Marine Provinces). In South Africa the distribution of this species has been confirmed using molecular data along the west and south-west coasts of South Africa: Port Nolloth to Muizenberg. Distribution records (based on morphological identification) exist for the Kei River region in the Eastern Cape (BOL 15252 & BOL 15257) and Langstrand, central Namibia (Lluch, 2002).

Habitat: Associated with kelp, either growing on the shell of the kelp limpet, *Cymbula compressa*, or (according to herbarium records) attached to the stipe of kelp. Commonly associated with *E. maxima*, but some specimens have been found on *L. pallida* (Greville) and *E. radiata* (C. Agardh) J. Agardh (Table 3.1). This species occurs in a shallow subtidal environment attached to kelp generally close to the surface but permanently submerged. Additionally, this species may be epiphytic on *Mazzaella capensis* (J. Agardh) Fredericq and *Chaetomorpha aerea* (Dillwyn) Kützing in the intertidal according to Lluch (2002).

Seasonality: Observations and collections were made throughout the year; however, it is not known if the species has a seasonal growth pattern.

Misapplied names: *Pyropia gardneri* (from South Africa, Stegenga *et al*., 1997).

Previously assigned codes, based on molecular data: *Pyropia* ZLI (Jones *et al*., 2004; Sutherland *et al*., 2011); *Pyropia* RSAk (Reddy *et al*., 2018).

Note: The morpho-anatomical characteristics of *Porphyra* sp. indet. (Stegenga *et al*., 1997) and *Porphyra* sp. (Lluch, 2002) agree with the current description of *Py. meridionalis*.

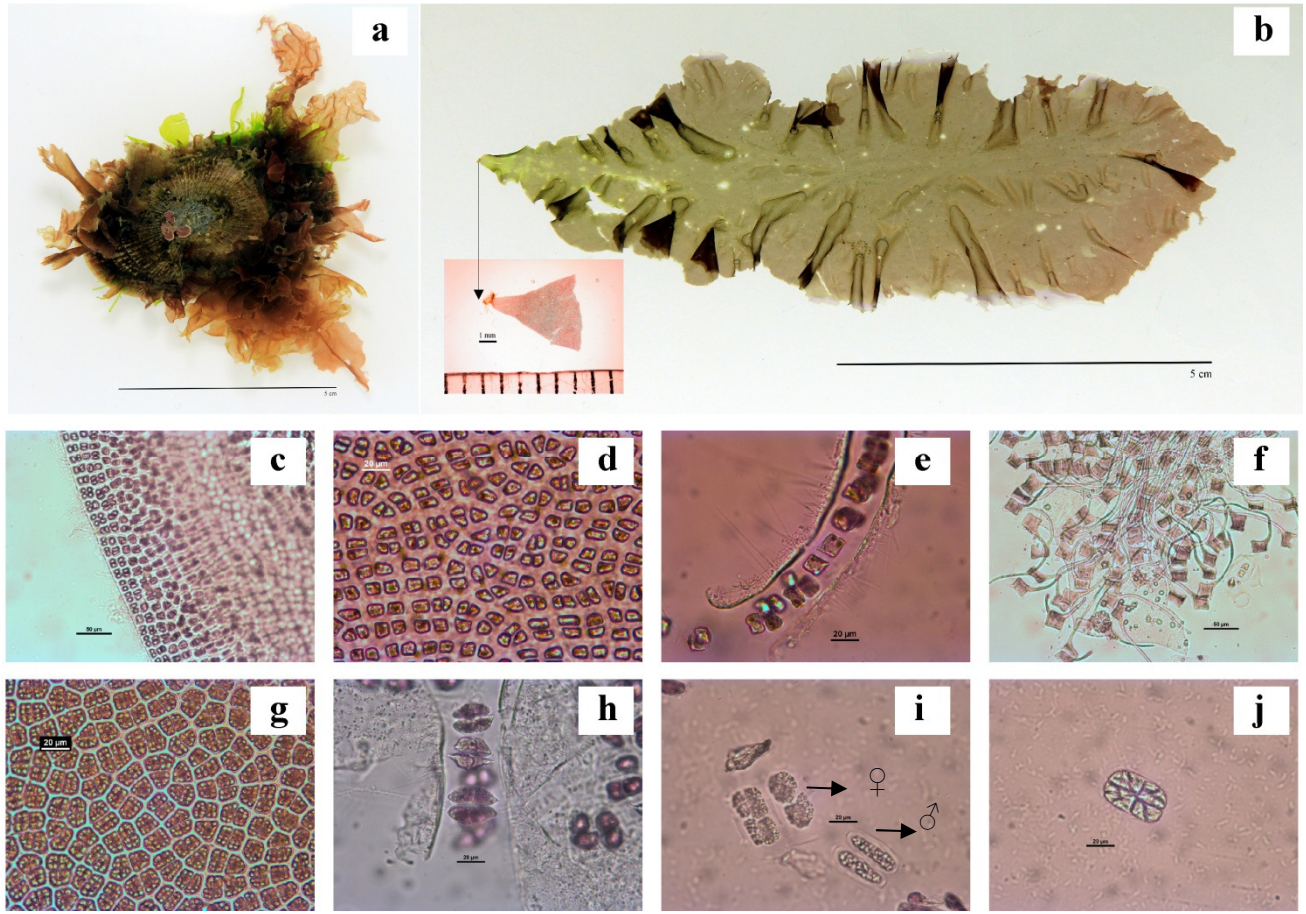

Fig. 3.1. Morphological and anatomical features of *Py. meridionalis* sp. nov. a) *Py. meridionalis* attached to its host *Cymbula compressa*; b) General morphology (holdfast inset); c) Surface view of cells along the thallus margin; d) Surface view of non-reproductive cells; e) non-reproductive in cross section of thallus, showing blade thickness; f) Rhizoidal cells near thallus base; g) Surface view of reproductive cells; h) Cross section of thallus showing female reproductive cells with distinct trichogynes on opposite ends; i) Female reproductive cells (larger & elliptical) and spermatia (lanceolate to fusiform); j) Zygotosporangium.

Below the description and distribution were extended for *Py. saldanhae* (Stegenga *et al*., 1997) and further details provided for this species as well as *Py. aeodis* (Griffin *et al*., 1999a). Additionally photographs of key anatomical features for both species were provided.

***Pyropia saldanhae* (Stegenga, J.J. Bolton & R.J. Anderson) J.E. Sutherland**

Basionym: *Porphyra saldanhae* **Stegenga, J.J. Bolton & R.J. Anderson.**

Habit: Gametophytes large lanceolate plants with highly undulate margins arising from an indistinct discoid holdfast; purple to deep maroon in colour with a patchwork of pinkish-red and yellowish-white fertile material visible around the margin. Base rounded or tapering with highly undulate margins near the base. Central part of thallus smooth, flattened and distinct from undulate margins. Fertile tissue seen as yellow to white streaks on a pinkish-red marginal band on the upper portion of thallus (toward the apex) which is generally paler in colour and has entire margins compared to the rest of the thallus. Depending on the size of the thallus, the apex may be extended or appear auriculate with entire margins. Fresh material dark purple becoming paler at apex, when dried retaining its rich colour and remaining darker at the base and lighter at the apex. Thalli generally large, ranging from 150–500 mm long and 40–50 mm wide for the lanceolate portion and as much as 100 mm wide at the widest points if auriculate. Blades monostromatic, 60–100 µm thick in cross section of thallus. non-reproductive cells in cross section 25–40 µm long and rectangular in shape, generally 2–3 times longer than wide. Cells with two stellate chloroplasts, each with a central pyrenoid, but specimens also observed with cells with a single chloroplast per cell. Non-reproductive in surface view arranged in pairs, creating longitudinal rows between cell pairs. In surface view both vegetative and reproductive cells arranged in longitudinal rows. Non-reproductive cells in surface view appear irregular in shape. Gametophytes monoecious, with cells rounded and in sets of 2 pairs; zygotosporangia deep red to maroon; spermatia yellow to golden. Spermatia smaller than zygotosporangia, ovate to lanceolate and made up of two groups of four tiers. Zygotospores ovate to round with 32 spores arranged in two tiers. Reproductive cells in cross section 40–45 µm long, spermatia roughly 4 times longer than wide, zygotosporangia as long as or only slightly longer than wide. Male and female reproductive cells intermingled, with islands of spermatangial sori scattered amongst zygotosporangial sori along the margins of the upper thalli.

Holotype: BOL 15255 (Yzerfontein, Bolton and Stegenga, 26/10/1988, Stegenga Sa 2098).

Representative DNA sequences: KY814935 (*cox*1), GU165838 (*rbc*L), AY292630 (nSSU).

Isotype: (Slide) BOL15255 (Yzerfontein, Bolton and Stegenga, 26/10/1988, Stegenga Sa 1039).

Type locality: Yzerfontein, Western Cape, South Africa.

Etymology: The name refers to Saldanha Bay, a large bay system on the west coast of South Africa where *Py. saldanhae* commonly occurs and that is in close geographic proximity to the type locality.

Distribution: South Africa and Namibia (Benguela Marine Province: Cape Peninsula, South Africa to Cape Fria in northern Namibia). This study provides new insights into the geographical distribution and possible range extension for this species further east to Rooiels, False Bay (100 km east of Olifantsbos). The distribution of this species has been confirmed (DNA) in the present study from Rooiels to Hondeklipbaai. Records of this species outside South Africa are based on morphological identification.

Habitat: This species is commonly epilithic in the lower eulittoral and sublittoral fringe but may grow on the stipes of *E. maxima*, generally on older kelps that have lost their secondary blades or that host a number of other epiphytes. Also found attached to mussels in the sublittoral fringe.

Seasonality: Occurs year-round (Dlaza 2011; this study).

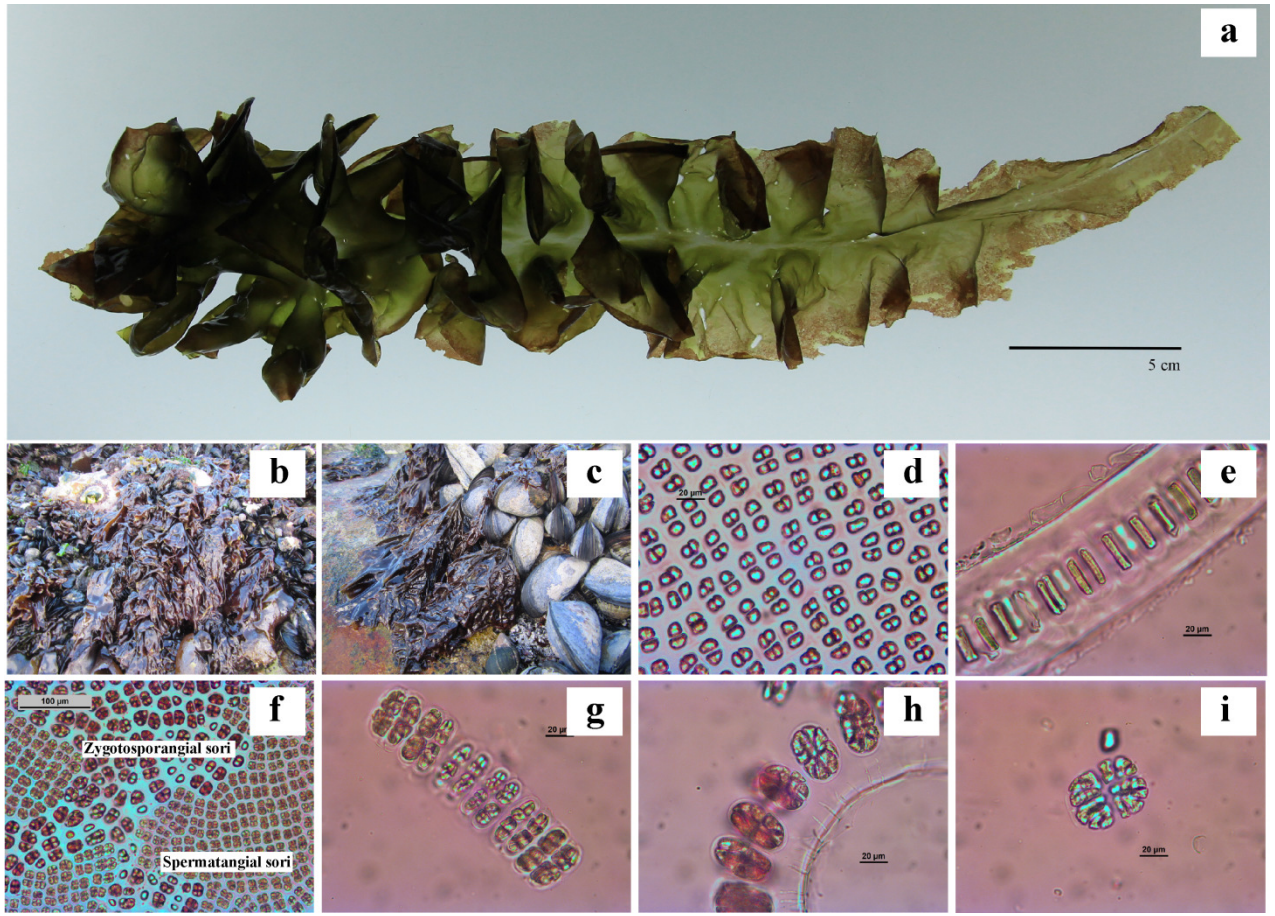

Fig. 3.2. Anatomical features of *Py. saldanhae* a) Thallus morphology; b) Plants usually occur in the sublittoral fringe amongst low-shore biota; c) Plants growing on mussels; d) Non-reproductive cells in surface view; e) Non-reproductive cells in cross section; f) Reproductive cells in surface view; g) Spermatia in cross section; h) Zygotosporangia in cross section; i) Zygotosporangium.

***Pyropia aeodis* (N.J. Griffin, J.J. Bolton & R.J. Anderson) J.E. Sutherland**

Basionym: *Porphyra aeodis* **N.J. Griffin, J.J. Bolton & R.J. Anderson**.

Habit: Thalli ovate, cordiform or umbilicate with reduced basal or central holdfast. Thallus centre smooth, flattened and thin; margins highly undulate. Fresh material olive green, green around the basal region, with brownish-red central thallus. Monoecious fertile rim around thalli (excluding basal portion) with irregular patches of pale white to yellow spermatial sori and pink to red zygotosporangial sori. Dried thalli remaining olive-green or brownish with a distinct fertile rim around thallus margin. May appear rosette in shape with distinct fertile margin when still attached to its host, *Pachymenia orbitosa*. Thalli generally small, ranging from 50 mm to 150 mm in length. Monostromatic blades 60–140 µm thick in cross section, generally thicker near the base. Non-reproductive cells in cross section 20–35 µm long by 8–10 µm wide, oblong to elliptical, generally 2–3 times longer than wide, with prismatic cells basally. Cells with two stellate chloroplasts, each with a central pyrenoid, but cells with single chloroplast also observed. Non-reproductive cells in surface view arranged in pairs and evenly distributed, creating longitudinal rows. Both non-reproductive and reproductive cells tightly packed in surface view. Monoecious; spermatia bright gold and zygotosporangia deep red to maroon, both fusiform but differing in size. Zygotosporangia larger, 65–100 µm by 25–40 µm, with two distinct prototrichogynes, spores arranged in 8–16 groups containing 32 spores. Spermatia 40–70 µm by 5–15 µm made up of two groups of four tiers and ovate to lanceolate. Patchwork of male and female reproductive cells around thallus margin.

Holotype: BOL 150073 (Kommetjie, Griffin, 16/05/1995, NJG-193).

Representative DNA sequences: KY814926 (*cox*1), GU165843 (*rbc*L), AY292624 (nSSU).

Isotype: BOL 150074 (Kommetjie, Griffin, 16/05/1995, NJG-190); BOL 150075 (Kommetjie, Griffin, 16/05/1995, NJG-191).

Type locality: Kommetjie, Western Cape, South Africa.

Etymology: *Py. aeodis* is epiphytic on *Pachymenia orbitosa* (formerly *Aeodes orbitosa*) after which it is named.

Distribution: South Africa and Namibia (Benguela Marine Province, from the Cape Peninsula in South Africa to northern Namibia). Its distribution has been confirmed, using molecular sequence data, along the south-western and west coast of South Africa, from Scarborough (Cape Peninsula) to Paternoster. Distribution records extending beyond this point and into northern Namibia are based on

morphology and substratum affinity (Griffin *et al*., 1999a) and BOL records: (Engledow, 13/04/1992, BOL 15279), (Engledow, 13/04/1992, BOL 15280), (Engledow, 17/03/1992, BOL 15260).

Habitat: Generally epiphytic on *Pachymenia orbitosa* in the low intertidal to sublittoral but may rarely be epilithic (based on personal observations made in late autumn & winter).

Seasonality: Summer annual, appearing in late spring, with the highest abundance and occurrence in summer and maturing in autumn. The growth pattern of this species closely matches the growth of its host, *Pachymenia aeodis* (Levitt *et al*., 1995).

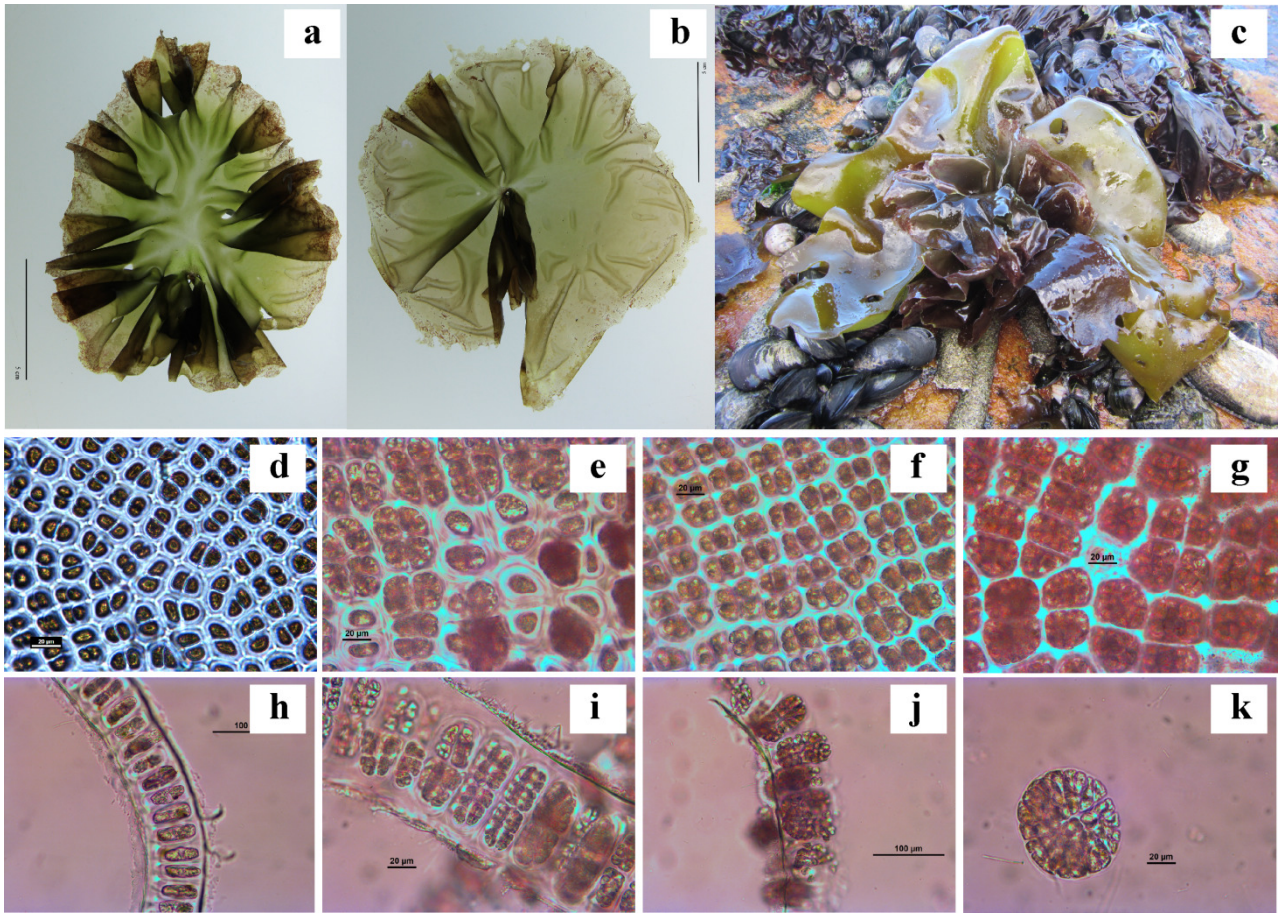

Fig. 3.3. Anatomical features of *Py. aeodis.* a) Morphology of thallus with basal holdfast; b) Morphology of thallus with central holdfast; c) Plants epiphytic on the larger, yellowish *Pachymenia orbitosa*; d) Surface view of non-reproductive cells; e) Surface view of intermingled reproductive and non-reproductive cells; f) Surface view of spermatial sori; g) Zygotosporangia in surface view; h) Non-reproductive cells in cross section of thallus; i) Spermatia in cross section of thallus; j) Zygotosporangia in cross section; k) Zygotosporangium.

Provisional identification

***Pyropia* cf. *suborbiculata* (Kjellman) Sutherland, Choi, Hwang & Nelson**

Basionym: *Porphyra suborbiculata* Kjellman.

Heterotypic synonym: *Porphyra carolinensis* Coll & J. Cox.

Habit: Single-bladed orbicular to irregularly shaped thalli, 20–40 mm long, maroon to purple with an indistinct holdfast. Monostromatic blades 20–35 µm thick. In cross section, non-reproductive cells rectangular to more rounded in appearance, 12–15 µm long by 10–15 µm wide, with distinct accentuated edges. In surface view, non-reproductive cells varying in shape and arranged haphazardly. Basal cells elongated. Reproductive cells ovate, larger, *ca*. 40 µm x 30 µm compared to non-reproductive cells. More rounded cells are presumably pre-mature female reproductive cells. Female reproductive cells without obvious trichogynes. No spermatangial sori were observed. Microscopic teeth protruding along the thallus margin, 10–20 µm with 1–3 cell layers and a triangular apical cell at the tip of each 'tooth'.

Type locality: Goto-retto, Nagasaki Prefecture, Japan.

Distribution: Widely spread (see Guiry & Guiry, 2018), confirmed by DNA sequence data as occurring on most major continental plates, however a number of distribution records have been based on morphological identification and require confirmation using DNA.

South African record: This record is mentioned in Stegenga *et al*. (1997), 15251 (BOL) as *P. carolinensis* (coll & det. H. Stegenga; 24/09/84) and was collected from Cape Infanta, attached to the wall of a man-made tidal pool.

Remarks: Morphological and cell characteristics (cell shape, size & agreement) agree with the description of *Py. suborbiculata*, particularly the diagnostic dentate margin along the thallus. However, no trichogynes were observed, which is a feature of *Py. suborbiculata*. This species is known to be monoecious, however it was difficult to confirm the sexuality of this specimen, or to successfully obtain DNA sequence data to confirm this identification. Nevertheless, this species has been recently introduced to several regions worldwide and its occurrence in South Africa cannot be ruled out.

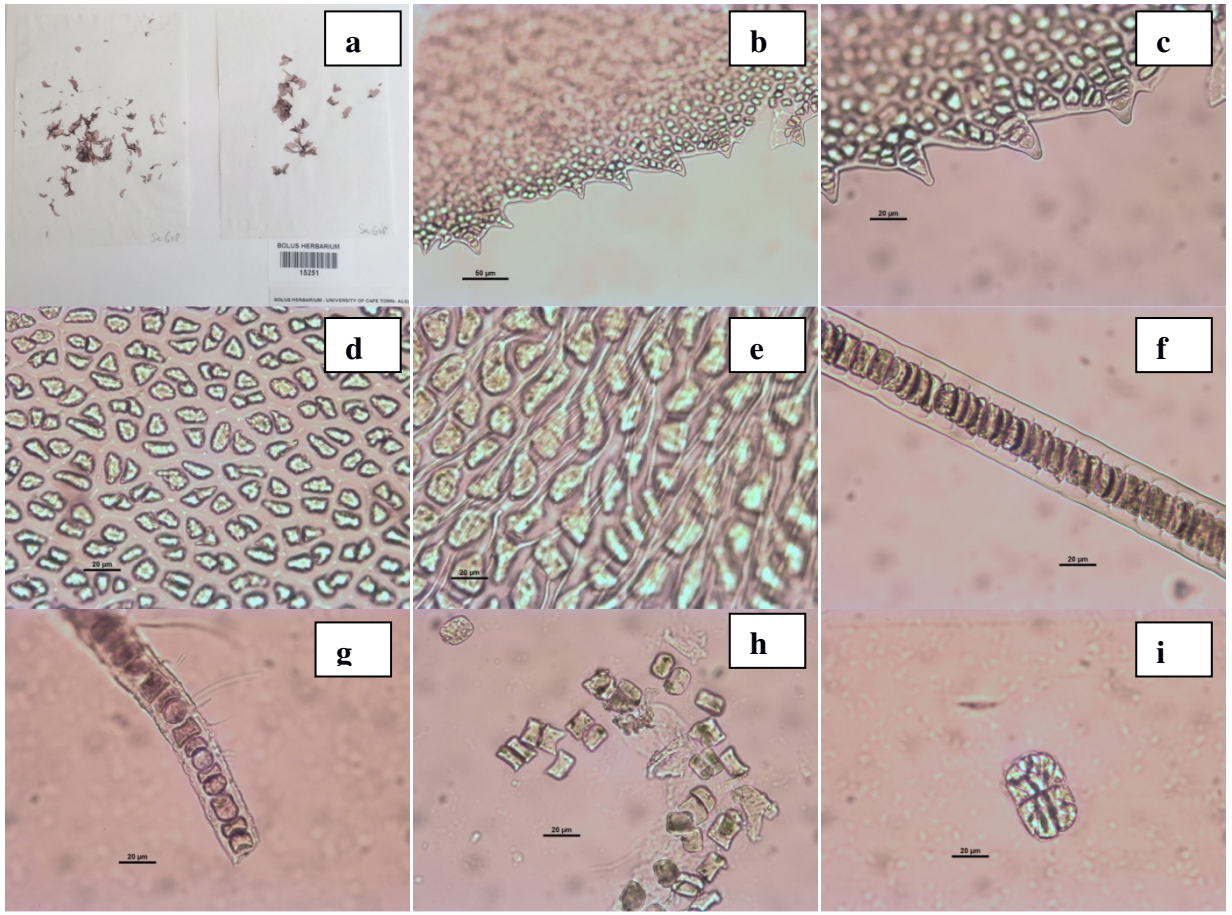

Fig. 3.4. Morphological and anatomical features of *Py*. cf. *suborbiculata* from South Africa a) Herbarium specimen15251 (BOL); b–c) Micro-dentation along the thallus margin; d) Non-reproductive cells in surface view; e) Rhizoidal cells at the base of thallus; f–g) Non-reproductive cells in cross section of thallus; h) Non-reproductive cells; i) Possibly endospore.

3.3.3. Phylogenetic relationships and affinities of southern African Pyropia

Sequences were generated only for new collections of *Pyropia* from South Africa. A total of 97 sequences were generated from a total of 44 specimens (26 new specimens from this chapter & 18 specimens from Chapter 2), for three unlinked loci (*cox*1, *rbc*L & nSSU). Sequence alignments for the *cox*1 gene were 669 bp long, for the *rbc*L gene 1411 bp and for the nSSU gene 1761 bp long, yielding a concatenated alignment of 4933 bp.

Species of South African *Pyropia* did not form a monophyletic group but were found in three separate clades spread out in the phylogram, and this pattern was consistent for all phylogenetic trees. *Pyropia meridionalis* is not closely related to the other two southern African endemic species of *Pyropia* or to other Southern Hemisphere species in this genus. Rather, it is sister to an undescribed species (6POR) represented by a single specimen collected from Texas in the Gulf of Mexico (Kucera & Saunders, 2012). These species are closely related based on the *cox*1 (4%) and *rbc*L (1%) genes but distinct based on DNA-based species delimitation (Supplementary fig. S3, S4). No comparisons could be made for the nSSU gene because this information is lacking for 6POR. *Pyropia meridionalis* and *Py*. 6POR are included in a Pacific-Atlantic molecular clade with distant relatives from the Northern Hemisphere (*cox*1: 11–12%, *rbc*L: 4–6%). Specimens that morphologically and anatomically resemble *Py*. *gardneri*, and that were reported from South Africa, were identified as *Py*. *meridionalis* based on sequence data. The true *Py*. *gardneri* is included in a completely separate and distantly related clade.

In contrast to *Py*. *meridionalis*, *Py*. *aeodis* and *Py*. *saldanhae* are included in clades shared with mostly other Southern Hemisphere species but differing in the degree of sequence divergence amongst related species. *Py. aeodis* shares a major clade with species predominantly from the Southern Hemisphere (New Zealand, Australia, the Falkland Islands and Antarctica) but also from the north Pacific (America). *Pyropia aeodis* is highly divergent from species found in Chile, the Falkland Islands and Antarctica (*cox*1: 7–10%, *rbc*L: 5–6%) and equally divergent or slightly more divergent (*cox*1: 8–10%, *rbc*L: 5–8%) from north Pacific species. *Pyropia saldanhae* shares a clade with species from the Falkland Islands and New Zealand and species within this clade are closely related (*rbc*L: 2%). *Pyropia pulchra* (formerly *Py*. *smithii*) occurs in the eastern north Pacific (Lindstrom & Hughey, 2016) and is placed sister (see gene/combination considered; Sutherland *et al*., 2011) to all Southern Hemisphere species in this clade and to which it is more distantly related (*cox*1: 8–10%, *rbc*L: 3%).

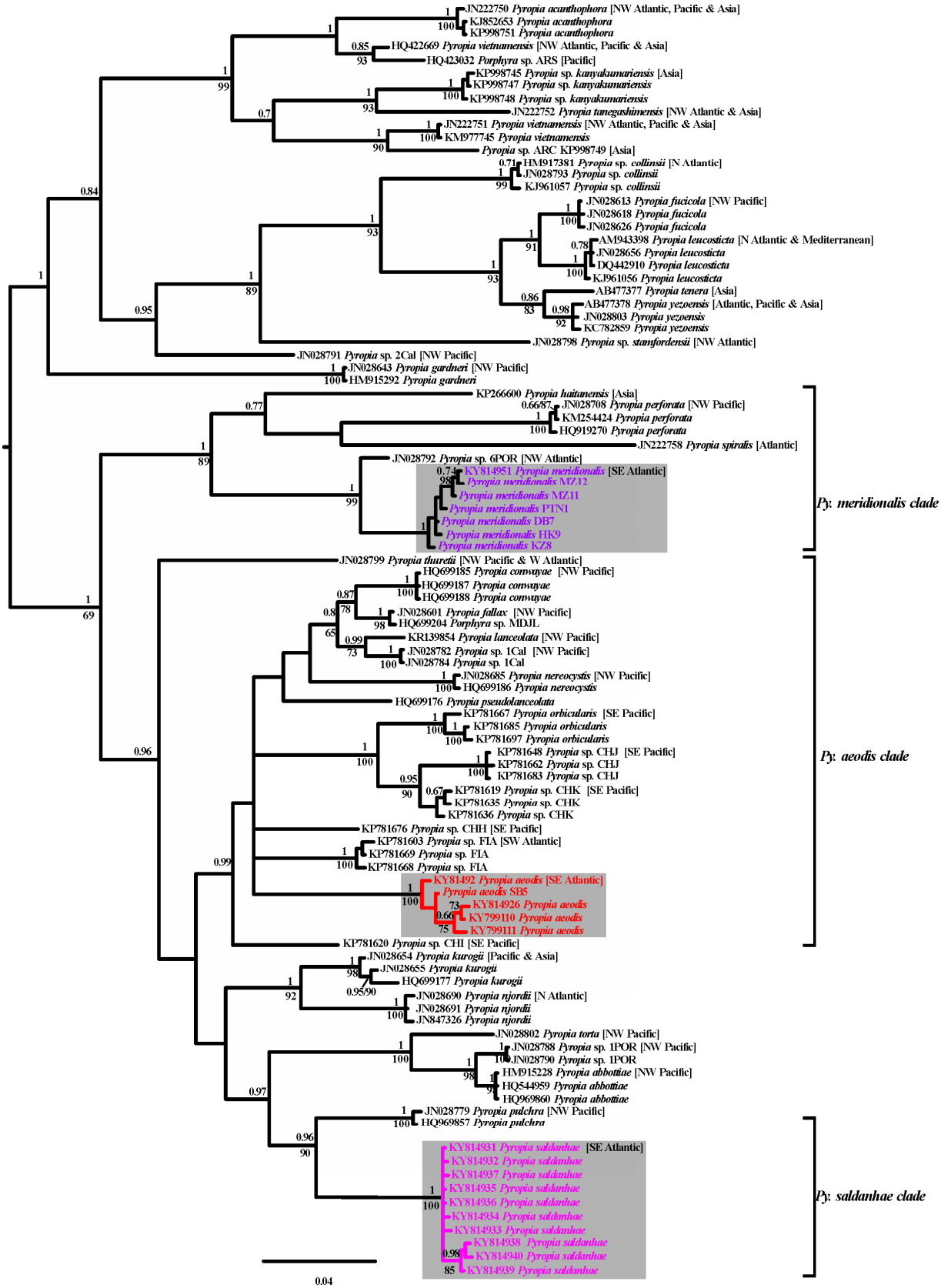

Fig. 3.5. Phylogram based on the *cox*1 barcoding region, PP indicated above nodal point and BP below and geographic region in square brackets.

Table 3.2. Summary of morphological, ecological and molecular traits of species of *Pyropia* recorded along the southern African coast. Taxonomic identities that have not been confirmed in this study (doubtful) are in grey and are provided for comparative purposes. Distributions are based on morphological and molecular records of the species.

Species	Blade thickness (µm)	Cell size (µm)	Cell Shape	Number of Chloroplast(s)	Mode of Reproduction	Thallus morphology	Thallus Size	Colour	Substratum	Depth	Seasonality	Distribution
Py. meridionalis	25–30 (60)	10–20	Square to rectangular	1	Monoecious	Lanceolate to obovate	Small	Deep ruby	Epizoic on *Cymbula compressa* or epiphytic on kelp	Subtidal	Austral summer	Muizenberg to Port Nolloth; the Kei River and Langstrand, Namibia
***Py. gardneri* (S.A)**	25–30	10–12	Square to rectangular	1	Monoecious	Lanceolate	Small	?	Epiphytic on *Ecklonia maxima*	Subtidal	?	Cape of Good Hope to Brandfontein
***Porphyra sp. indet.* (Stegenga *et al*., 1997)**	40–60	20–25	Square to rectangular	1	Monoecious	Lanceolate to obovate	Large	?	Epiphytic on *Ecklonia maxima* and *Laminaria pallida*	Subtidal	?	Platboombaai to Yzerfontein
***Porphyra sp.* (Lluch, 2002)**	24–36	14–20	Square to rectangular	1	Unknown	Elongate to obvate	Small	?	Epiphytic on *Mazzaella capensis* and *Chaetomorpha aerea*	Eulittoral	?	Langstrand, Namibia
***Py.* cf. *suborbiculata* (S.A)**	20–35	10–15	Square to rectangular	1	Monoecious?	Orbicular	Small	Pale pink	Epilithic	Intertidal	?	Cape Infanta
Py. aeodis	60–140	25–35	Ellipse	2	Monoecious	Cordate	Small to medium	Red-Purple	Epiphytic on *Pachymenia orbitosa*	Sublittoral fringe	Late austral spring to autumn	Cape peninsula to west coast of South Africa; Namibia and southern Angola
Py. saldanhae	60–100	25–40	Rectangular to ellipse	2	Monoecious	Lanceolate	Medium to large	Purple	Epilithic	Sublittoral fringe	Year-round	Rooiels to Hondeklipbaai; Namibia and southern Angola

3.3.4. Key to the South African species of **Pyropia**

1. a. Blade thickness less than 60 µm, cells with one chloroplast, cells square to rectangular, non-reproductive cells with distinct trichogynes.....2

   b. Blade thickness greater than 60 µm, cells with two chloroplasts, non-reproductive cells elliptical to rectangular.....3

2. a. Epilithic; thalli orbicular; microscopic teeth along the thallus margin.....***Py*. cf. *suborbiculata***

   b. Epibiont on kelp (epiphytic on kelp or epizoic on kelp limpets); thalli lanceolate to obovate, ruby red to deep purple; thallus margin entire (no microscopic teeth).....***Py*. *meridionalis***

3. a. Epilithic; thalli lanceolate with highly undulated margins, purple; non-reproductive cells rectangular.....***Py*. *saldanhae***

   b. Epiphytic on *Pachymenia orbitosa*; thalli cordate to obovate, brownish-red; non-reproductive elliptical.....***Py*. *aeodis***

NB: non-reproductive cells refer to cells without propagules

3.4. DISCUSSION

Prior to this study, seven species of *Pyropia* (as *Porphyra*) were recorded from southern Africa. In the present study three of these species are validated using an integrative taxonomic approach based on morphological, ecological and molecular data. These are: *Py. saldanhae*, *Py. aeodis*, *Py. meridionalis* sp. nov. and a fourth species is tentatively identified as the widely distributed *Py. suborbiculata* based on morphology. However, given the taxonomic uncertainty and limited information regarding *Py.* cf. *suborbiculata* from South Africa, comparisons between this species and the three southern African endemic species mentioned above are limited. The identities of *Pyropia gardneri* and *Porphyra* sp. indet. from South Africa (sensu Stegenga *et al*., 1997), and *Porphyra* sp. (Lluch, 2002) from Namibia, are discussed.

3.4.1. Pyropia meridionalis, *a new species from southern Africa*

Py. meridionalis is newly described from the temperate coastline of southern African. This study provides evidence that *Py. meridionalis* was previously misidentified as *Py. gardneri* from South Africa by Stegenga *et al*. (1997). *Pyropia* sp. indet. and *Py. gardneri* from South Africa were considered to be separate entities, because they differed by minor morpho-anatomical characteristics (Stegenga *et al*., 1997). *Pyropia gardneri* was noted to have a smaller, lanceolate thallus and recognizable trichogynes, while *Porphyra* sp. indet. had a larger, cordate thallus and marginally larger blade thickness (Stegenga *et al*., 1997). Many anatomical features, and to a lesser extent morphological features such as the distinctive stipe-like holdfast and two-sectored fertile bands along the thallus margin, overlap with the current description of *Py. meridionalis*. Herbarium specimens indicate that *Py.* sp. indet. and *Py. meridionalis* (as *Py. gardneri*; Stegenga *et al*., 1997) appear to represent individuals at either end of the size spectrum of a single species. The Namibian entity described by Lluch (2002) is of a size intermediate between the entities described by Stegenga *et al*. (1997) as *Py.* sp. indet and *Py. gardneri*, and thus fits within the range of *Py. meridionalis*. Therefore, *Porphyra* sp. indet (Stegenga *et al*., 1997) and *Porphyra* sp. (Lluch, 2002) are now merged into *Py. meridionalis* based on morphological analyses in the present study. *Py. meridionalis* was later identified as a genetically distinct entity ZLI (Jones *et al*., 2004) which was presumed to be conspecific with 'RSAk' (Chapter 2). In the present study these entities are confirmed to be conspecific using additional sequence data. Therefore, the molecular entities RSAk and ZLI, as well as the morphologically identified, *Py. gardneri* (Stegenga *et al*., 1997) and *Porphyra* sp. indet. (Stegenga *et al*., 1997) from South Africa, and *Porphyra* sp. (Lluch, 2002) from Namibia are subsumed into *Py. meridionalis*.

Pyropia meridionalis is a kelp-associated species that is commonly found on the kelp limpet, *Cymbula compressa*, or on the stipes of *Ecklonia maxima*, and rarely on other species of southern African kelp, *Laminaria pallida* and *E. radiata* or other algae. This species occurs along the south-west and west coast of South Africa throughout the year, but may extend further north to Langstrand, Namibia (Lluch, 2002), and further east to the Kei River region on the south-east coast of South Africa. Its distribution thus spans across two marine provinces, the warm-temperate Agulhas Marine Province and the cool-temperate Benguela Marine Province. Although specimens collected during this study were associated with kelp attached at *ca.* 2–3 m in depth at low tide, blades were generally found attached to the kelp limpet/kelp closer to the surface. The diminutive bladelets of *Py. meridionalis* may be easily missed in the field because kelp and the kelp limpet, *Cymbula compressa* commonly host a number of other bladed red algal species (Anderson *et al*., 2006). As such this species has likely been overlooked until recently and may also be overlooked in other areas of southern Africa.

New collections, as well as an older specimen of *Py. meridionalis* (as *Porphyra* ZLI) collected by Jones *et al*. (2004), were supplemented with herbarium records (dating back 60 years) of specimens that fit the description of *Py. meridionalis*. All new collections (this study) were epizoic on the kelp limpet, *C. compressa*. However, herbarium collections suggest that *Py. meridionalis* may also occur attached to the stipes of *E. maxima* and also rarely on the stipes of *E. radiata* and *Laminaria pallida* or even other algae (Lluch, 2002).

The supplemental use of herbarium specimens in the present study aided in determining the geographical distribution range, seasonality and possible hosts for this species, indicating the importance of such records (Brodie *et al*., 2007, 2008b; Nelson *et al*., 2013; Gunnarsson *et al.*, 2016).

Pyropia meridionalis is distinct from other southern African endemic species of *Pyropia* in various aspects. In the field, these algae are readily distinguished based on morphology and substratum. Although *Py. saldanhae* has occasionally been found growing subtidally on *E. maxima* or epizoically on mussels, it occurs mainly on rock in the lower sublittoral fringe. When *Py. meridionalis* and *Py. saldanhae* co-occur on kelp they are easily distinguished by gross thallus morphology, such as the shape and size of the thallus as well as morpho-anatomically. *Pyropia saldanhae* has a larger, deep-maroon thallus with highly ruffled margins while the much smaller blades of *Py. meridionalis* are more variable in colour and have somewhat undulated to entire margins. Furthermore, the non-reproductive cell characteristics of *Py. meridionalis* are distinct; cells are small and square to rectangular in shape and differ from the relatively large, rectangular to elliptical cells common in *Py. saldanhae* and *Py. aeodis*. Even though all three southern African endemic species of *Pyropia* are monocieous, *Py. meridionalis* lacks the characteristic intermingling of male and female reproductive

cells often visible along the thallus margin in the other species. Instead, spermatial sori or zygotosporangial sori appear as distinct sectored margins and developing female reproductive cells (with bipolar trichogynes) are situated toward the inner parts of the thallus.

3.4.2. Habitat as a taxonomically informative character for species of southern African **Pyropia**

All three southern African species of *Pyropia* are highly divergent in their general habitat preference: *Py. meridionalis* is a subtidal kelp epibiont, *Py. saldanhae* is commonly epilithic in the lower eulittoral and *Py. aeodis* is epiphytic on *Pachymenia orbitosa*. Habitat is therefore a taxonomically informative character for southern African *Pyropia*. Similarly, habitat preference has been found to be taxonomically informative for species of *Pyropia* in Japan (Miyata & Kikuchi, 1997). A distinct habitat association allows for easy identification of species along with anatomical and morphological verification, and limits the need for molecular identification which can be costly and may not always be convenient. It is also possible that habitat preference may be an evolutionary conserved trait for southern African *Pyropia*, whereby species have evolved to specifically occur on certain substrata. However, this will require further study.

Pyropia *species associated with kelp*

In addition to *Py. meridionalis*, other species, such as *Py. drachii*, *Py. gardneri* and *Py. nereocystis*, have been reported to be epiphytic on kelp in other regions of the world. *Pyropia drachii* occurs in France and Britain and grows epiphytically on *Laminaria hyperborea* (Gunnerus) Foslie. The affinity of this species to *Py. meridionalis* is currently unknown. The diminutive blades of *Py. gardneri* resemble those of *Py. meridionalis*, and indeed the latter species was previously misidentified in South Africa as *Py. gardneri* (Stegenga *et al*., 1997). *Pyropia gardneri* grows epiphytically on the blades of *Laminaria setchellii* P.C. Silva and *Egregia menziesii* (Turner) Areschoug, and appears to be restricted to Pacific North and Central America based on sequence data. The much larger, *Py. nereocystis* (C.L. Anderson) S.C. Lindstrom, grows up to a few metres in length and occurs along the eastern Aleutian Islands to central California. This species grows epiphytically on *Nereocystis leutkeana* (K. Mertens) Postels & Ruprecht but may also occur rarely on other species of kelp in the region. Another bladed bangialean species, *Wildemania amplissima* (Setchell & Hus) S.C. Lindstrom, which is a common component of the macroalgae on the west coast of North America, commonly grows epilithically but may grow on *Nereocystis leutkeana*, occasionally; however, these algae are distinguished from *Py. nereocystis* based on overall morphology and anatomy (Proudfoot & Fretwell, 2015). This situation is similar to *Py. meridionalis* and *Py. saldanhae* co-inhabiting *E. maxima*.

3.4.3. Endemism in the Benguela Marine Province in southern African

Although, *Py*. *saldanhae*, *Py*. *aeodis* and *Py*. *meridionalis* are known to occur in the Benguela Marine Province based on morphological identification, their distribution has only been confirmed (DNA) in South Africa (Jones *et al*., 2004; Chapter 2). Specimens identified as *Py*. *saldanhae* from Namibia differed from South African specimens in cell dimensions and their epiphytic nature (Lluch, 2002). Similarly, the identification of *Py*. *aeodis* in Namibia was based on its morphology and substratum affinity alone (Griffin *et al*., 1999a; BOL records). The Namibian distributions for these species therefore remain to be confirmed by molecular studies.

Species boundaries for *Py*. *saldanhae* and *Py*. *aeodis* in South Africa were largely confirmed using DNA-based species delimitation methods (Chapter 2), although high intraspecific diversities in these species were noted. This is particularly true for *Py*. *aeodis* for which a more comprehensive and targeted sampling strategy is required in order to fully understand the genetic diversity and geographic distribution of this species. In the present study, blades were observed growing in spring, abundantly in summer and extending into autumn (Chapter 2, this study). This pattern closely resembles that of its host *Pachymenia orbitosa* (Levitt *et al*., 1995). The generally epiphytic *Py*. *aeodis* was found to occur epilithically, in late autumn and early winter which could be related to the scarcity or absence of its host.

The distribution range of *Py*. *saldanhae* was extended (previously known from Olifantsbos to Hondeklip Bay) further east into False Bay (Rooiels) where it was observed for three consecutive years, but only during the winter. *Pyropia saldanhae* has never previously been found east of the Cape Peninsula despite extensive seaweed surveys along the south-western Cape region, including False Bay (Stegenga *et al*., 1997). Thus, we cannot rule out the possibility that the species' range has recently shifted eastward which would coincide with observations for the kelp, *E. maxima* (range shift) and rock lobster, *Jasus lalandii* (biomass shift) (Bolton *et al*., 2012; Blamey & Branch, 2012; Blamey *et al*., 2015). Despite some differences in the gross thallus morphology of plants found in Rooiels, anatomical characteristics overlapped with the current description of *Py*. *saldanhae*. This was supported by molecular sequence data and DNA-based species delimitation methods (Chapter 2) with levels of divergence within the range currently accepted in other species of Bangiales (*rbc*L: 1%).

3.4.4. Taxonomic uncertainties

The anatomical features of specimens identified as *Py*. cf. *suborbiculata* are mostly consistent with the description of this species, with the most striking feature being microscopic teeth along the thallus margin (Neefus *et al*., 2008; Verges *et al*., 2013a). Although many common characteristics are shared between *Py*. *meridionalis* and *Py*. cf. *suborbiculata* from South Africa, they primarily differ in

substratum preference and micro-dentation along the thallus margin of the latter, which has not been recorded for any other species of Bangiales from South Africa. Microscopic teeth along the thallus margin however are not unique to *Py. suborbiculata* and have also been recorded in other, albeit not as widely distributed, Asiatic species such as *Pyropia tanegashimensis* and *Pyropia vietnamensis*.

Pyropia suborbiculata was first described from Japan and has since been recorded along the coasts of North America (Atlantic), Mexico (Pacific), the Iberian Peninsula (Atlantic Ocean & Mediterranean Sea), Brazil, Australia and New Zealand (Broom *et al*., 2002; Monotilla & Notoya, 2004; Milstein & Oliveira, 2005; Tsutsui *et al*., 2005; Neefus *et al*., 2008; Verges *et al*., 2013a). Molecular data have confirmed that *Py. suborbiculata* is widespread and that it has been recently introduced in many parts of the globe, but may be overlooked because of its small thalli (Neefus *et al*., 2008; Verges *et al*., 2013a). Where *Pyropia suborbiculata* has been introduced it tends to be widely distributed, however, in South Africa it has only been collected on a single occasion.

Nevertheless, it is difficult to confirm the identity of *Py.* cf. *suborbiculata* based on morphological and anatomical features alone. In the absence of new collections it would be necessary to sequence fragments of herbarium specimens to confidently identify this species. Unfortunately the existing specimens were first preserved using formalin (Rob Anderson personal communication 2016) and are therefore not suitable for DNA analyses.

3.4.5. Phylogenetic relationships of species of **Pyropia** *endemic to southern Africa*

Southern African *Pyropia* do not form a monophyletic group when placed in a global phylogeny and this suggests that each species probably colonised and speciated along this coast independently.

Pyropia meridionalis was resolved in a clade with high species diversity in the Pacific region (see fig. S4). Within this clade, *Py. meridionalis* and 6POR are very closely related and further study is required to determine if these species are conspecific or sibling species that have recently diverged. However, according to available data presented in Chapter 2 *Py. meridionalis* and 6POR are currently retained as separate species. Based on the shallow sequence divergence a recent split between these northern (6POR) and southern (*Py. meridionalis*) Atlantic sister species is hypothesised. However, in the absence of a calibrated molecular clock for the bladed Bangiales it is not possible to estimate a time for such an event.

The unusual placement of *Py. meridionalis* in a Pacific-Atlantic clade raises the question as to whether this species is truly endemic to southern Africa. As an example, *Py. acanthophora* Oliveiria & Coll was first described in Brazil in 1975, but was only later recorded in the Pacific where it is native. *Pyropia acanthophora* has since been shown, using a molecular approach (Milstein *et al*.,

2012, 2015; Dumilag *et al*., 2016), as a species introduced to Brazil from the Pacific. Similarly, Nelson *et al*. (2014) recently recorded *Py*. *koreana* in New Zealand. This species was originally described from Korea but Nelson *et al*. (2014) suggest it may be native to some other region in the world. However, despite the possibility of *Py*. *meridionalis* having a centre of diversity or origin elsewhere in the world, it is currently only known from southern Africa.

In the larger clade in which *Py*. *saldanhae* is placed, it is closely related to species from the Falkland Islands and New Zealand and much more distantly related to a species (*Py*. *pulchra*) from the north Pacific. This suggests a recent divergence between these Southern Hemisphere taxa and an earlier isolation and divergence from Northern Hemisphere taxa. *Pyropia aeodis* is resolved in a major clade with species predominantly from the Southern Hemisphere but also a few species from the Northern Hemisphere. The high species diversity and deep divergence between taxa in this clade, particularly between Southern Hemisphere taxa, suggest that species belonging to this clade have been established in the Southern Hemisphere for a long time.

Phylogenetic relationships between *Py*. *saldanhae* and its closely related Southern Hemisphere relatives, and *Py*. *aeodis* and its affinities to related Southern Hemisphere taxa, support the notion of past connectivity in the Southern Ocean. Hommersand (1986) proposed a model whereby long range dispersal of marine organisms may have been facilitated by the West Wind Drift in the Southern Hemisphere in the late Miocene (*ca*. 5–12 MYA). The later strengthening of the West Wind Drift may have then isolated populations, which subsequently speciated along various Southern Hemisphere coastlines such as South America, South Africa, Australia, and New Zealand (Hommersand, 1986). Aspects of the model have since been supported by molecular phylogenies for a number of taxa (Coyer *et al*., 2001; Hommersand & Fredericq, 2003; Russell *et al*., 2009). However, to date no study has provided a time-calibrated phylogeny for Southern Hemisphere connectivity and therefore the age of these events remains uncertain. Nevertheless, molecular phylogenetic data of siphonous green algae suggest a more recent isolation for species in this group of green algae which could have occurred in the late Pleistocene-Pliocene, rather than the Miocene (Verbruggen *et al*., 2005). Even though a timescale for the evolution of *Pyropia* is lacking, data from the present study suggest that dispersal and speciation in the temperate *Py*. *saldanhae* and the temperate *Py*. *aeodis* clade took place at different periods. This suggests a more complex temporal scenario for past and present dispersal in the Southern Ocean (Hommersand, 1986; Rothman *et al*., 2015). However, further research is required for much of the Southern Ocean islands and Antarctica in order to further clarify phylogenetic relationships and biogeographic affinities of species within these clades.

3.5. CONCLUSION

This chapter assessed the diversity of *Pyropia* in southern Africa, including species that were previously assigned to the genus *Porphyra* or existed in the literature based only on sequence information. The number of species documented in southern Africa has been reduced from six to four: *Py*. *saldanhae*, *Py*. *aeodis*, *Py*. *meridionalis* sp. nov and *Py*. cf. *suborbiculata*. The identity of the last-named species is based on morpho-anatomical characters alone and requires molecular confirmation. Species boundaries were confirmed for *Py*. *saldanhae* and *Py*. *aeodis* and new information provided on their descriptions, distributions and ecology. The morphological entities previously recorded in South Africa as *Py*. *gardneri*, *Py*. sp. indet (Stegenga *et al*., 1997) and *Porphyra* sp. (Lluch, 2002), and the genetic entities ZLI (Jones *et al*., 2004), and RSAk (Reddy *et al*., 2018), are now subsumed into *Py*. *meridionalis* sp. nov. This study demonstrates a case of taxonomic inflation that has been resolved using molecular data. *Py*. *meridionalis* adds to the ever-growing list of *Pyropia* species globally and is the third endemic species of *Pyropia* known from the Benguela Marine Province in southern Africa. The new species is easily distinguished in the field from the other endemic species based on morphology and its association with kelp. In the present study new collections were supplemented with herbarium records and demonstrate the value of such records in studies of species discovery (Brodie *et al*., 2007, 2008b; Nelson *et al*., 2013). This study also highlights the possibility of undocumented seaweed diversity in subtidal habitats where the algal flora is under-studied and other new species may be awaiting discovery.

CHAPTER 4

Cryptic Speciation and Biogeographical Structure in the Genus *Porphyra* from Temperate South Africa, Including the Description of *Porphyra agulhensis* sp. nov

4.1. INTRODUCTION

Cryptic species are morphologically similar, but genetically or physiologically distinct entities (Mayr, 1963; Henry, 1985; Knowlton, 1986). They exist in almost all branches of the tree of life (Pfenninger & Schwenk, 2007) and are particularly common in marine organisms with simple morphologies such as corals (Ladner & Palumbi, 2012) and algae (Zuccarello & West, 2003; Saunders, 2008; Payo *et al*., 2013; Muangmai *et al*., 2014). In algae, the recent application of Molecular-Assisted Alpha Taxonomy (MAAT) which is the application of DNA barcodes in species discovery, routinely followed by morpho-anatomical analyses, has uncovered extensive cryptic diversity within various macroalgal groups (Saunders, 2005; Cianciola *et al*., 2010; Hind *et al*., 2014; Schneider *et al*., 2015).

The morphologically simple red algal order, the Bangiales, was initially classified into two genera based on morphology. However, a revision of the order using molecular sequence data found that this classification did not reflect the extensive diversity in the Bangiales, and 15 genera were recognized (Sutherland *et al*., 2011). A large majority of the almost 130 (Sutherland *et al*., 2011) to 150 (Brodie *et al*., 2008a) species belonging to the genus *Porphyra* were reassigned to new or resurrected genera (Sutherland *et al*., 2011). The once speciose '*Porphyra*' retained only eight named species and a suite of unnamed species, half of those that were named had been described in the 1800s as morphospecies. With the aid of molecular sequence data, many additional species in the Northern Hemisphere were unmasked from some of these morphospecies. As a result, new species were described based on molecular and morphological characters, such as *Porphyra mumfordii* (Lindstrom & Cole, 1992c), *P. dioica* (Brodie & Irvine, 1997) and *P. corallicola* (Kucera & Saunders, 2012). More recently, extensive species diversity based on molecular sequence data has been found in the Southern Hemisphere, where many new and endemic species have been found along the coasts of Chile (Guillemin *et al*., 2016), South Africa (Jones *et al*., 2004, Chapter 2) and New Zealand (Broom *et al*., 2004, 2010, Sutherland *et al*., 2011). Despite this, no new species have been described from the Southern Hemisphere, and at present only two names, which have been confirmed using molecular data, exist for the region: *P. capensis* in South Africa (Milstein *et al*., 2005 but see Chapter 2) and *P. lucasii* in Australia (Farr *et al*., 2003; Sutherland *et al*., 2011).

4.1.1. Taxonomy of southern African Bangiales

Porphyra was first recorded from the South African coast in 1843 and two morphological species were described: the rosette shaped *Porphyra capensis* and the lanceolate *P. augustinae* nom. illeg. (Kützing, 1843). The latter was later synonymized with *P. capensis* in 1883 by J. Agardh.

P. capensis has been recorded as extending further north along the west African coastlines of Namibia (Lawson *et al*., 1975; Wynne, 1986; Lluch, 2002) and Angola (John *et al.*, 1979) in the south-east

Atlantic Ocean. However, Anderson *et al*. (2009) recorded an unknown species of *Porphyra* in southern Angola which they did not specify was *P. capensis*. The larger Atlantic Ocean distribution is reported to include various subantarctic islands: Fuegia, Iles Kerguelen, Auckland Islands and Campbell Island (Papenfuss, 1964) as well as Tristan and Gough Islands (Chamberlain, 1965) and the temperate coastline of Argentina (Pujals, 1963) in the south-west Atlantic Ocean. *Porphyra capensis* has also been recorded from St. Paul Island (Silva *et al*., 1996) in the south Indian Ocean and along the temperate coastline of Chile (Ramírez & Santelices, 1991) in the south-east Pacific Ocean. All records of *P. capensis* mentioned above have been based on morphological identification.

In 2004, a preliminary biodiversity assessment of the Bangiales using the nuclear nSSU gene suggested a much higher species diversity of *Porphyra* along the South African coastline which they referred to as the 'Cape cluster' (Jones *et al*., 2004). This was largely confirmed and additional diversity was recognized using more extensive gene and taxon sampling, and the application of various DNA-based species delimitation methods (Chapter 2). The latter study showed that South African *Porphyra* comprised 10 species, which were likely endemic to South Africa/southern Africa or at least the South Atlantic Ocean. This was in agreement with a recent molecular study that could not confirm the presence of bona fide *P. capensis* (Ramírez & Santelices, 1991) along the Chilean coast (Guillemin *et al*., 2016), reinforcing the notion that distribution records of *P. capensis* based on morphology alone can be misleading, and that the occurrence of this species complex outside South Africa needs to be verified.

4.1.2. Phylogeography along the South African coast

The application of molecular markers is not only routinely used to identify species or cryptic species, but has also been valuable for studying their distribution patterns in time and space. Phylogeography is the study of spatial patterns of gene flow within a species or among species within a complex or across multiple species (Avise, 2000; Avise *et al*., 1987). Phylogeographic patterns shared across taxa with common distribution ranges are often associated with historic or environmental processes that impede or promote gene flow (Avise *et al*., 1987; Bermingham & Moritz, 1998; Bernatchez & Wilson, 1998; Avise, 2000; Beheregaray, 2008). These patterns can therefore offer insights into mechanisms driving divergence and speciation. In the marine environment, climatic oscillation and oceanographic processes, such as currents or upwelling, play a major role in shaping genetic patterns (Muller *et al*., 2012; Reynolds *et al*., 2014; Toms *et al*., 2014; Li *et al*., 2016).

The genus *Porphyra* in South Africa exhibits a large degree of variation in thallus form, texture, colour, reproductive strategy (monoecious, dioecious or androdioecious) and populations are widely distributed throughout the intertidal, although a major dominance generally occurs in the upper eulittoral. Three distinct forms have been documented throughout the distribution range of this genus,

which spans ~2000 km (Isaac, 1957; Graves, 1969). Rosette and lanceolate forms occur along the west coast and a dwarf form along the south and south-east coasts (Isaac, 1957; Graves, 1969). *Porphyra* is absent further east along the South African coast (personal observation) where seawater temperature rapidly increases (Smit *et al*., 2013). Seawater temperature is generally considered a major factor controlling the geographical distribution of seaweeds (Smit *et al*., 2017).

Porphyra's extensive distribution occurs along a dynamic coastline situated between two ocean basins and influenced by contrasting current regimes. The warm Agulhas current in the Indian Ocean flows south- and westward along the east and south coasts of South Africa, and the cold Benguela current in the Atlantic Ocean basin flows northward along the west coast of southern Africa (Isaac, 1937; Shannon, 1985). The Benguela/Agulhas transition zone has been shown to act as a strong genetic barrier for closely related species and lineages within species (von der Heyden *et al*., 2009; Teske *et al*., 2011a). Additionally, along the west coast of South Africa, south-easterly winds inshore of the Benguela current result in coastal upwelling throughout the region (Andrews & Hutchings 1980, Waldron & Probyn, 1992). Upwelling is temporally and spatially heterogeneous along the South African coast (Hutchings *et al*., 2009) with five major seasonal upwelling cells (Lutjeharms & Meeuwis, 1987; Fig. 4.5). A near permanent upwelling cell occurs along the South African/Namibian border and cells decrease in intensity to the north and south of this region (Lutjeharms & Meeuwis, 1987). Upwelling has been shown to act as a vicariant barrier to gene flow in various marine animals in southern Africa (Muller *et al*., 2012; Henriques *et al*., 2014, 2016; Reid *et al*., 2016) and for coastal marine animals (Waters & Roy, 2004; Veale & Lavery, 2011) and algae (Lourenco *et al*., 2016) in other regions.

The genus *Porphyra* along the South African coastline is an ideal candidate to test patterns of speciation in relation to its extensive spatial distribution and highly variable morphology. The present study had three aims;

a) To determine whether molecular species defined in Chapter 2 have diverged in morphological, anatomical or ecological traits. Specifically, to investigate whether different forms (dwarf form, rosette & lanceolate) could be matched to different molecular species using two different methods (classical and contemporary taxonomy).

b) To describe molecular entities that are distinguishable based on distinct morpho-anatomical traits as new species and document the known distribution ranges of all molecular species, and to determine whether certain molecular species are limited to particular geographic regions.

c) To assess the phylogeographic structure of selected species of *Porphyra* along the South African coast and identify processes impacting evolutionary relationships within and among molecular species.

4.2. MATERIALS AND METHODS

4.2.1. Sampling

Approximately 250 specimens representing different morphological variants of *Porphyra* were collected from various substrata and from different shore positions from 35 sites (Fig. 2.1) spanning the entire distribution range of the genus in South Africa (Table S1). Site names mentioned in this chapter can be found in Fig. 2.1. Thalli were rinsed using seawater to remove epiphytes. Basic information was noted in the field such as habitat, the colour of fresh specimens, texture and general thallus morphology, and key diagnostic features were photographed. Length and width were measured using a calliper or tape measure, depending on the size of the thallus, and the position of the holdfast was recorded. Herbarium voucher specimens were prepared, a fragment of the thallus including reproductive tissue (when present) was preserved in 5% formalin/seawater for anatomical examination and another fragment was preserved in silica gel for DNA analyses.

4.2.2. Herbarium specimens

The type specimen of *Porphyra capensis* was not available for examination and was therefore only examined online. All South African specimens of *Porphyra* in BOL were re-examined (Table 4.1). Information from specimens that resembled any new and morphologically distinct species was included to this study. However, no herbarium specimens were sequenced (discussed later) or sectioned (no morpho-anatomical measurements). Nevertheless, information from herbarium records aided in elucidating distribution ranges and seasonal patterns/occurrences for new morphologically distinct species.

4.2.3. Morpho-anatomical analysis

Formalin samples were rinsed with seawater before preparing slides and thin hand sections were cut under a dissecting microscope using a scalpel. Sections were water-mounted on slides and sealed using cover slips. Slides were viewed under a Leica Wild M10 light microscope equipped with an Olympus D50 digital camera and key features were photographed. The highly pigmented cells found in fresh or preserved *Porphyra* limited the need for staining of material. For a representative set of samples for each species, photographs were taken in surface view and cross sections of the thalli for non-reproductive and reproductive cells (when the latter were present).

Morpho-anatomical characters

Morpho-anatomical characters were examined for selected specimens (subsets) representing each of the ten molecular species identified in Chapter 2. Since each molecular species was represented in the collection by a different number of specimens, five from each molecular species were selected, spanning the distribution range of that molecular species.

Quantitative and qualitative morpho-anatomical characters were taken into consideration. Quantitative characters for morphology were thallus length, width and shape (L/W ratio, used as an indication of the general shape, e.g. rosette or lanceolate). Qualitative characters for morphology were colour, texture and position of the holdfast. The anatomical quantitative characters analysed were blade thickness, vegetative (non-reproductive) cell height and width and (when present) reproductive cell height, width and number of spores. Qualitative characters for anatomy were cell shape and colour as well as the arrangement of non-reproductive and reproductive cells. Ecological characters included substratum, shore position and geographic distribution (Table 4.1).

For a large majority of specimens morpho-anatomical features were indistinct or overlapped with one another. For these species, morpho-anatomical characters were analysed using two statistical approaches:

a) Testing for distinct clusters based on morpho-anatomical and ecological data (independent of genetic data) using non-metric Multidimensional Scaling (nMDS) and,

b) Testing for species boundaries (diagnostic or non-overlapping measurements) using *a priori* species groups, as defined by genetic data (Chapter 2), using Discriminant Analyses (DA) and ANOVA.

Resultant morpho-anatomical clusters were visually compared with genetic clusters (nMDS plot) to determine if morpho-anatomical and ecological features were congruent with genetic differences.

Morphological characters were also analysed for all specimens (n = ~250) but results were similar to those achieved using a subset of the dataset (five plants from across the distribution range per each of the 10 molecular species according to Chapter 2), so the former results are not presented.

Table 4.1. Morpho-anatomical and ecological features analysed for specimens of *Porphyra* from South Africa. QT = Quantitative and QL = Qualitative.

Morphology		**Anatomy**				**Ecology**		
		Non-Reproductive		**Reproductive**				
QT(mm)	QL	QT(µm)	QL	QT(µm)	QL	Substratum	Shore position	Distribution
Thallus length	Colour	Blade thickness	Shape	Blade thickness	Shape and size of cells	Rock	High	West coast
Thallus width	Texture	Cell height	Number of chloroplast per cell	Cell height	Presence of trichogynes	Plant	Mid	South coast
Size ratio (L/W)	Holdfast	Cell width	Arrangement	Cell width	Arrangement and number of spores	Animal	Low	

Statistical analyses

All statistical analyses were run in R (R, Core Team, 2017) using the packages Vegan, Calibrate and GGplot2. For the first approach, quantitative and qualitative data were analysed separately and features such as the general shape (rosette or lanceolate) or molecular clade were subsequently added to the clusters on the nMDS diagram (Fig. 4.4). Data were standardized prior to analyses, and goodness of fit tests run to check if assumptions were met. Reproductive characters (blade thickness, height and width of cells) were analysed separately, as not all thalli collected were in a reproductive state.

For the second approach, specimens were grouped according to genetic species clusters, and a Discriminant Analysis (DA) was run. Additionally, a series of ANOVAs were used to test for differences among selected characters across species, and a post-hoc test (Tukey HSD) was used to determine which species pairs differed for characters that were significantly different. The median and ranges of these characters for each molecular species were visualized using box and whisker plots. In both approaches all features were initially included and then an optimality criterion was applied, whereby features were hierarchically excluded, and the analyses re-run. For example, in an analysis that initially included five features, the succeeding run included four features and the run after that included three features. This was done to ensure that any patterns were not masked by noise in the data (features that may be common across species).

4.2.4. Molecular analyses

DNA isolation, PCR amplification and sequencing followed the methods of Chapter 2 & 3. New sequences were generated for the nSSU gene for representative samples from each of the molecular species defined in Chapter 2. Additional sequences for this gene from South African *Porphyra* were obtained from GenBank. Sequence data for the *cox*1 and *rbc*L genes from Chapter 2 were added to

the new dataset in the present to generate a concatenated alignment (nSSU, *rbc*L, *cox*1), and included sequences from closely related species obtained from GenBank following the literature and a BLAST search.

Alignments were edited and aligned using the ClustalW function in BioEdit (Hall, 1999) and Jmodeltest v 2.1.10 (Posada, 2008) was used to select the most appropriate evolutionary model for each gene. Phylogenetic trees were reconstructed using Bayesian Inference (BI) in MrBayes v. 3.2.6 (Ronquist & Huelsenbeck, 2003) and maximum likelihood (ML) with Randomized Accelerated Maximum Likelihood (RAxML) for web servers (Stamatakis, 2006; Stamatakis *et al*., 2008). For BI trees, two concurrent runs were implemented, each of five million generations, sampling every 1000 trees under thermally controlled chains (two hot & two cold). Each run and combined runs were checked for convergence using Tracer v. 1.5 (Rambaut & Drummond, 2014). Combined trees from each run were used to reconstruct a 50% majority rule tree after 25% of the trees were discarded; posterior probability values were calculated from the remaining trees. RAxML trees were run using a gamma rate heterogeneity model (GTR) and all other settings left at default.

Genetic diversity and distribution of the genus* Porphyra *in South Africa

Haplotype networks were reconstructed to visualise the genetic diversity within and among closely related species in relation to their geographical distribution. Haplotype networks were generated in R (R, Core Team, 2017) using the package Pegas (Paradis, 2010). Only the *cox*1 networks are presented because a) they illustrate biogeographic patterns better than the slower evolving *rbc*L or nSSU genes (Payo *et al*., 2013; Guillemin *et al*., 2016) and b) a larger number of sequences were available for this gene compared to the other two. Uncorrected pairwise genetic distances were calculated in MEGA v. 6.0 (Tamura *et al*., 2013).

All specimens that were successfully sequenced were included in the genetic diversity analyses and did not follow the subset selection of specimens used in the morpho-anatomical analyses. Basic diversity indices were calculated in DNAsp (Librado & Rozas, 2009) for the full dataset for all species containing more than five individuals and neutrality tests were performed using the same program for each zone for species selected for phylogeographic analyses (detailed below).

Genetic diversity was considered in relation to temperature differences associated with different currents/oceans and in relation to upwelling (detailed below). Coastal temperature profiles for the South African coast were generated based on *in situ* measurements. This was chosen over temperature profiles generated using offshore satellite data because the latter have been shown to inaccurately represent the coastal environment, particularly in upwelling regions of the South African west coast (Smit *et al*., 2013). Monthly mean temperatures were obtained from https://robert-

schlegel.shinyapps.io/SACTN and data points were extrapolated to represent the entire coastline. Maps were generated in R using the packages GGplot2 (Wickham, 2016) and Viridis (Garnier, 2017).

Existing oceanographic, biological and geological information were used to identify areas of the coastline influenced by major oceanographic features that could impact gene flow, such as upwelling along the west coast of South Africa (Lutjeharms & Meeuwis, 1987). Additionally, the bathymetry of the coastline was used as an indication of the paleocoastline. Genetic patterns (variation and distribution) of species of South African *Porphyra* as well as intraspecific phylogeographic patterns were related to these features.

Comparative phylogeography and demographic history

Selection of species

The *cox*1 and *rbc*L genes were used to infer past demographic changes, genetic structure and the evolutionary history of selected species (those with adequate sample sizes for phylogeographic inferences).

Division of coastline

The South African coastline was divided into zones based on the location of known semi-permanent (seasonal) upwelling cells described by Lutjeharms and Meeuwis (1987), which were hypothesized to act as potential genetic barriers. Based on this scheme the coastline was divided into seven regions; two major and two minor upwelling regions (Zones) along the west and south-west coasts and three non-upwelling regions along the south-west and south coasts of South Africa. It should be noted that upwelling occurs sporadically in certain sections of the designated 'non-upwelling regions', however it is much less intense than the major seasonal upwelling patterns present along the west and south-west coasts of South Africa.

The effect of upwelling on the genetic structure of species of *Porphyra* along the coast of South Africa was tested using an Analysis of Molecular Variance (AMOVA) implemented in Genalex v 6.5 (Peakall & Smouse, 2006). For each species, individuals were grouped into populations based on their distribution, for example all individuals belonging to the genetic species RSAa collected from Zone 1 were treated as a population. Heat maps were generated in R using the package NMF (Gaujoux *et al*., 2010). These were used to visualize pairwise phiPT values between regions for each species. PhiPT is a measure of genetic differentiation and is presented on a scale from 0–1, values approaching 1 indicate genetic structure. Mismatch distribution (MMD) plots calculated in Arlequin (Excoffier & Lischer, 2010) were reconstructed for each region (upwelling & non-upwelling zones)

for each species and redrawn in R (R, Core Team). MMD rather than Bayesian skyline plots were used as there is no reliable mutation rate for the *cox*1 or *rbc*L gene for *Porphyra*. Furthermore, it was not possible to use mutation rates from other red algae because the relative mutation rates of mtDNA, nucDNA and ptDNA are different in *Porphyra* compared to other red algae (Smith *et al*., 2012).

Table 4.2. Division of the South African coast into upwelling and non-upwelling regions following Lutjeharms and Meeuwis (1987).

Delineation	Sites	Upwelling	Upwelling cell	Upwelling activity (%)
Zone 1	Port Nolloth, Kleinzee, Hondeklipbaai	Major upwelling zone	Namaqua	60
Zone 2	Doringbaai, Lamberts Bay, Elandsbaai	Major upwelling zone	Columbine	40
Zone 3	All sites on the west coast of the Cape Peninsula	Minor upwelling zone	Peninsula	10
FB (False Bay)	Buffels Bay, Miller's Point, Glencairn, Muizenberg, Strand, Rooiels	Non-upwelling zone	N/A	N/A
EOCH (East of Cape Hangklip)	De Kelders, Vermont, Pearly Beach	Non-upwelling zone	N/A	N/A
Zone 4: (Suiderstand/Cape Agulhas)	Suiderstand	Minor upwelling zone	Agulhas	10
SC (South coast)	Mossel Bay to Natures Valley	Non-upwelling zone	N/A	N/A
SEC (South-east coast)	Cape St. Francis to Port Alfred	Non-upwelling zone	N/A	N/A

4.3. RESULTS

4.3.1. A re-description of the genus **Porphyra** *C. Agardh in South Africa*

Gametophyte thalli bladed, membranous and monostromatic. Blades lanceolate, rosette (umbilicate) or orbicular and ranging in size from a few mm to more than a metre (only to about 30 cm on the south coast). Gametophyte phase alternating with a conchocelis-phase: a shell-boring microscopic stage that germinates into filaments. *Porphyra* can be monoecious, dioecious or androdioecious (hermaphrodites) and is able to reproduce sexually or asexually (in both the bladed and conchocelis-phase), via various types of spores. Female reproductive cells typically without trichogynes except in one species, *P. agulhensis* (described below). Modes of reproduction may vary among species and may even differ within a single species found in different localities. Gametophytes found growing on a range of substrata (animals, marine algae & rock), generally in the eulittoral zone but may also occur subtidally.

4.3.2. Species descriptions

Two molecular species (RSAi & RSAj) of *Porphyra* delimited in Chapter 2 were morphologically and anatomically indistinct from one another but were distinct from all other molecular species (RSAa–h) from South Africa. The more abundant and widely distributed of the pair (RSAi) is described below as a new species and RSAj recognized as its cryptic sister species. RSAi and RSAj were retained as two separate molecular species using a concatenated tree (Fig. 2.2) as well as single gene trees (Figs. S1 & S3). These molecular species (RSAi and RSAj) differ genetically from other South African *Porphyra* by 4–5% in the *cox*1 gene, 2–3% in the *rbc*L gene and 1–2% in the nSSU gene. The term duo (i.e. a pair) is adopted to collectively refer to RSAi and RSAj. The term species duo is used similar to the term species complex.

Porphyra agulhensis sp. nov. **M.M. Reddy, J.J. Bolton & R.J. Anderson**

Misapplied name: *P. capensis* (Isaac, 1957; Graves, 1969).

Previously assigned code based on molecular data: *Porphyra* ZPP (Jones *et al*., 2004), *Porphyra* RSAi (Reddy *et al*., 2018).

Holotype: BOL201161 (Port Elizabeth, Reddy, 08/07/2015, PE12).

Epitypes: BOL201162 (Port Alfred, Reddy, 07/07/2015, PA3/D2270); BOL201163 (Mossel Bay, Reddy, 09/07/2015, MB3/D2219).

Type locality: Port Elizabeth 33°58'50"S 25°39'34"E.

Etymology: This species is named for the Agulhas Marine Province where it is endemic.

Diagnosis: Deeply laciniate rosette thalli consisting of multiple lanceolate to orbicular blades with entire margins attached to a central discoid holdfast. The number of blades and degree of laciniation varies among individuals with some specimens exhibiting a star-shaped appearance. Thallus delicate and often curled up on itself. Thalli generally small, blades commonly around 60 mm in length but ranging from 20 mm to 300 mm. Fresh specimens light olive green to light pinkish-brown, progressively becoming more green or golden brown, but remaining light-coloured when dry. Dried specimens adhering poorly to herbarium sheets.

Blades monostromatic, 60–65 (–75) µm thick. In cross section of the thallus, vegetative cells 25–40 (–60) µm high, commonly around 30 µm, rectangular to ellipsoid, and 5–10 µm wide. Cells with a single stellate chloroplast. In surface view, non-reproductive and reproductive cells evenly distributed in longitudinal rows with large spaces between neighbouring cells. Zygotosporangia 35–65 µm in size and slightly larger than non-reproductive cells. Spermatia small, lanceolate and bright yellow. Zygotosporangia large, ovate and deep maroon. Female reproductive cells with recognisable bipolar trichogynes. Spermatia arranged in 8 rows, in two columns, in pairs (16–32 tiers). Zygotosporangia arranged in four columns (32–64 tiers). Monocieous habit in fertile plants.

Habitat: Rocky substrata along the mid to high eulittoral. Occurring year-round but more common and abundant in summer, occurring in dense stands (Fig. 4.1).

Fig. 4.1. *Porphyra agulhensis* sp. nov. at Keurboomstrand (close to Natures Valley) growing epilithically in a dense stand during the austral summer. Photo credit: Sam Bolton.

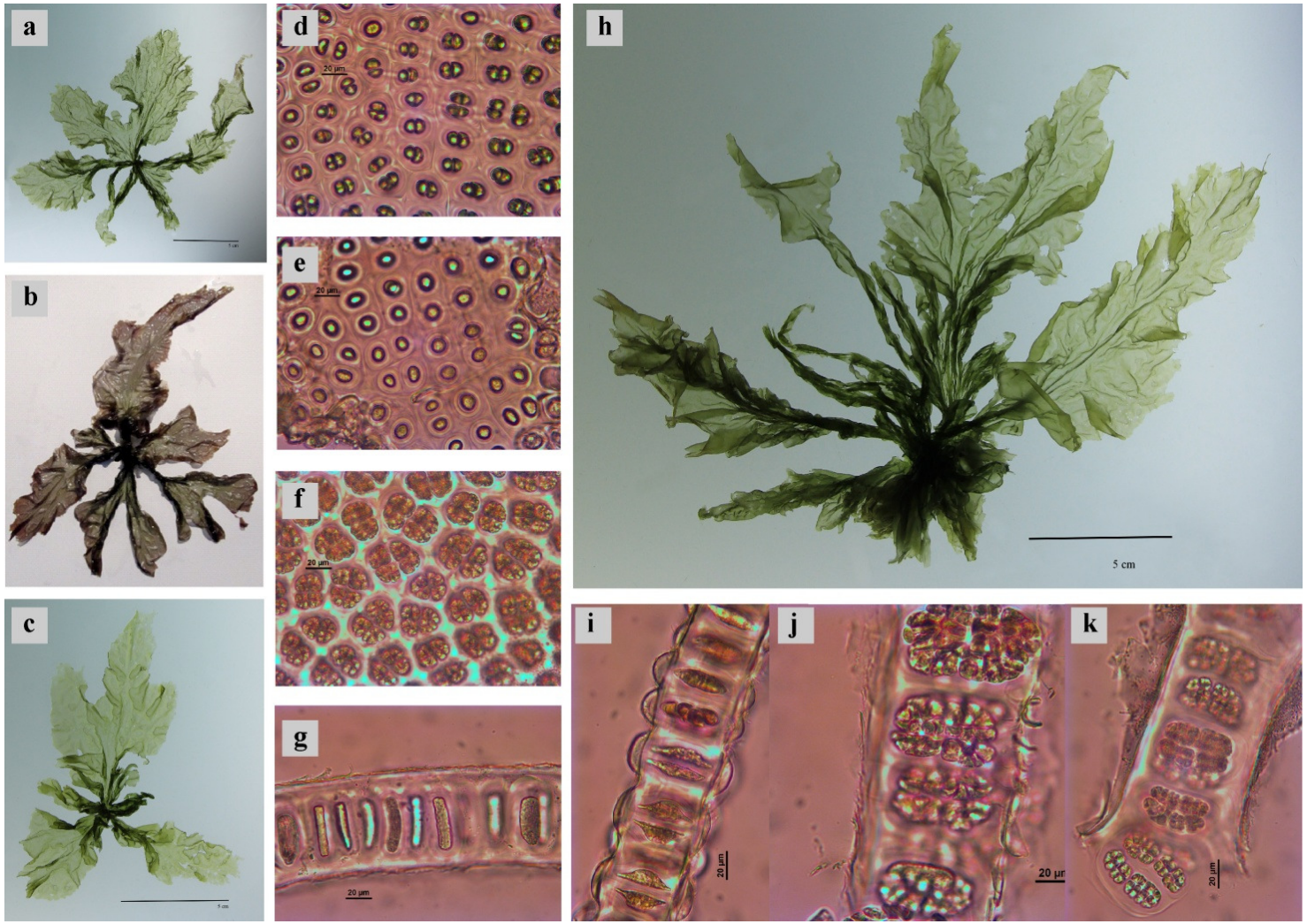

Fig. 4.2. *Porphyra agulhensis* sp. nov. Morphological and anatomical characters. a–c, h) Overall thallus morphology, d) Cells diving; surface view, e) Non-reproductive cells in surface view, f) Surface view showing zygotosporangia, g) Cross section of thallus showing non-reproductive cells with one chloroplast per cell, i) Cross section of thallus showing immature and developing reproductive cells, j) Zygotosporangia in cross section and k) Spermatangia in cross section.

***Porphyra* RSAj**

Identification: Morphologically indistinct but molecularly distinct from *P. agulhensis* sp. nov. This species is currently regarded as a cryptic sister species and at present is only distinguished from *P. agulhensis* sp. nov. using molecular data.

Specimens: EL1–7, DKG5.

Distribution: Endemic to the South African coast, found in East London, and De Kelders *ca.* 1000 km west. These two sites lie just outside the present distribution range of *P. agulhensis* sp. nov.

Note: This species is rare, being sparsely distributed in East London and only collected on one occasion from De Kelders. This species was found along the mid-eulittoral zone at both sites (East London and De Kelders).

Table 4.3. Specimens of *Porphyra agulhensis* collected from the South African coast.

New collections							
Location	**Co-ordinates**	**Substratum**	**Season (Austral)**	**ID and date**	**Ref no.**	**Collector**	**Remarks**
Mossel Bay	-34.183329, 22.157912	Epilithic	Winter	MB1–4, 09/07/2015	BOL201163	Reddy	(MB3) Epitype
Herald's Bay	-34.055565, 22.388327	Epilithic	Winter	HB1–4, 09/07/2015		Reddy	
Knysna, Buffels Bay	-34.085241, 22.959587	Epilithic	Winter	BBK1–4, 09/07/2015		Reddy	
Knysna, Heads	-34.076339, 23.060099	Epilithic	Winter	KH1–5, 09/07/2015		Reddy	
Nature's Valley	-33.986831, 23.547905	Epilithic	Winter	NV1, 06/12/2014		Anderson	
Plettenberg Bay	-34.059958, 23.380328	Epilithic	Winter	PBB1–12, 08/07/2015		Reddy	
Cape St. Francis	-34.207477, 24.835742	Epilithic	Winter	CSF1–8, 08/07/2015		Reddy	
Port Elizabeth	-33.976426, 25.650168	Epilithic	Winter	PE1–13, 08/07/2015	BOL201161	Reddy	Holotype (PE12)
Port Alfred	-33.603654, 26.901147	Epilithic	Winter	PA1–6, 07/07/2015	BOL201162	Reddy	Epitype (PA3)
Port Alfred, Breakwater	-33.603654, 26.901147	Epilithic	Summer	Jan–Feb 2001	N/A	Jones *et al*. 2004	As *Porphyra* ZPP956 (AY292636)
Location	**Co-ordinates**	**Substratum**	**Season (Austral)**	**Date**	**Ref no.**	**Collector**	**Remarks**
Brandfontein (West of Cape Agulhas)	-34.7670, 19.8670	Epilithic, high intertidal	Summer	11/11/1989	BOL 15275	Bolton & Stegenga	
Jongensfontein	-34.433, 21.333	N/A	Spring	16/10/2001	BOL 15300	Stegenga	
Vleesbaai	-34.290546, 21.914587	N/A	Spring	17/10/2001	BOL 15302	Stegenga	
Keurboomstrand	-34.004757,	Suprlittoral, isolated	Winter	11/06/1987	BOL	Stegenga	

	23.457111	rock on sandy beach			15294		
Natures Valley, Blue rocks	-33.988343, 23.548062	High intertidal	Spring	18/10/1997	BOL 15337	Bolton & Stegenga	
Natures Valley	-33.988343, 23.548062	N/A	Spring	14/04/1994	BOL 15324	Bolton & Stegenga	
Tsitsikamma	-34.019993, 23.857272	High intertidal	Spring	16/10/1997	BOL 15323	Bolton & Stegenga	
Tsitsikamma	-34.019993, 23.857272	Intertidal	Spring	17/10/1997	BOL XX	Anderson & Bolton	
Tsitsikamma	-34.019993, 23.857272	High intertidal	Spring	16/10/1997	BOL XX	Bolton & Stegenga	
Tsitsikamma	-34.019993, 23.857272	High intertidal	Spring	16/10/1997	BOL XX	Bolton & Stegenga	
Tsitsikamma	-34.019993, 23.857272	High intertidal, including rockpools	Spring	15/10/1997	BOL XX	Bolton & Stegenga	
Tsitsikamma, Storms River Mouth	-34.023134, 23.899217	Intertidal	Spring	17/10/1997	BOL XX	Anderson & Bolton	
Tsitsikamma, Storms River Mouth	-34.023134, 23.899217	Intertidal	Spring	17/10/1997	BOL BOL 15338	Anderson & Bolton	
Storms Rover Mouth	-34.023134, 23.899217	High to mid shore	Summer	XX/01-02/2001	WELT A23091	Jones *et al*. 2004	As *Porphyra* ZPP956
Cape Padrone	-33.772240, 26.468066	N/A	Spring	26/10/2003	BOL 15298	Stegenga	
Cannon Rocks	-33.749351, 26.549862	High intertidal – Supratidal	Spring	24/10/2003	BOL 15299	Stegenga	
Kenton-on-sea	-33.618678, 26.879139	High intertidal to supratidal	Summer	02/12/1987	BOL 15290	Stegenga	
Kenton-on-sea	-33.618678, 26.879139	High to mid shore	Summer	X/01-02/2001	WELT A23093	Jones *et al*. 2004	As *Porphyra* ZPP956
Port Alfred	-33.603654, 26.901147	Epilithic, upper intertidal	Autumn	18/03/1987	BOL 15296	Stegenga	
Port Alfred	-33.603654, 26.901147	High to mid shore	Summer	01-02/2001	WELT A23094	Jones *et al*. 2004	As *Porphyra* ZPP956

East London	-33.018732, 27.920927	High intertidal	Spring	19/11/1987	BOL 15291	Stegenga
Mkambathi	-31.289242, 30.013430	N/A	Spring	07/10/2002	BOL 15303	Stegenga

- XX= missing information

The Porphyra capensis *complex*

The remaining specimens represented eight molecular species (RSAa–h) and were distributed from Cape Agulhas to Port Nolloth. Morphological and anatomical characteristics of these molecular species were within the range of the current description of *P. capensis*. These species were therefore referred to as the *Porphyra capensis* complex.

Morpho-anatomical data (nMDS plots) were incongruent with molecular species clusters when applied to all specimens (n = ~250) for quantitative and qualitative analyses (not shown) or when applied to a subset of specimens (n = ~30). No clear groupings were recovered from the nMDS plots which showed that morpho-anatomical features from lanceolate and rosette forms overlapped, as did morpho-anatomical and ecological features from different molecular species (Fig. 4.4). Morpho-anatomical clusters and molecular species clusters were also incongruent even when an optimality criterion was applied, i.e. as features were hierarchically excluded (not shown).

Similarly, no clear groupings were recovered from DA analyses. Box and whisker plots showed that the range of various morpho-anatomical characters generally overlapped between molecular species in the subset (Fig. 4.3) and for the full dataset (not shown). This was further supported as no statistical difference was found between various characters among different molecular, species except for blade thickness (Table 4.4). Tukey tests indicate that the two species that differed in bladed thickness were RSAc and RSAd, which were represented by one and two specimens respectively.

An example is shown for at least one analysis from each of the two approaches a) no *priori* assignments (nMDS) and b) *a priori* assignment according to genetic data (ANOVA) (Fig. 4.3 & 4.4; Table 4.1).

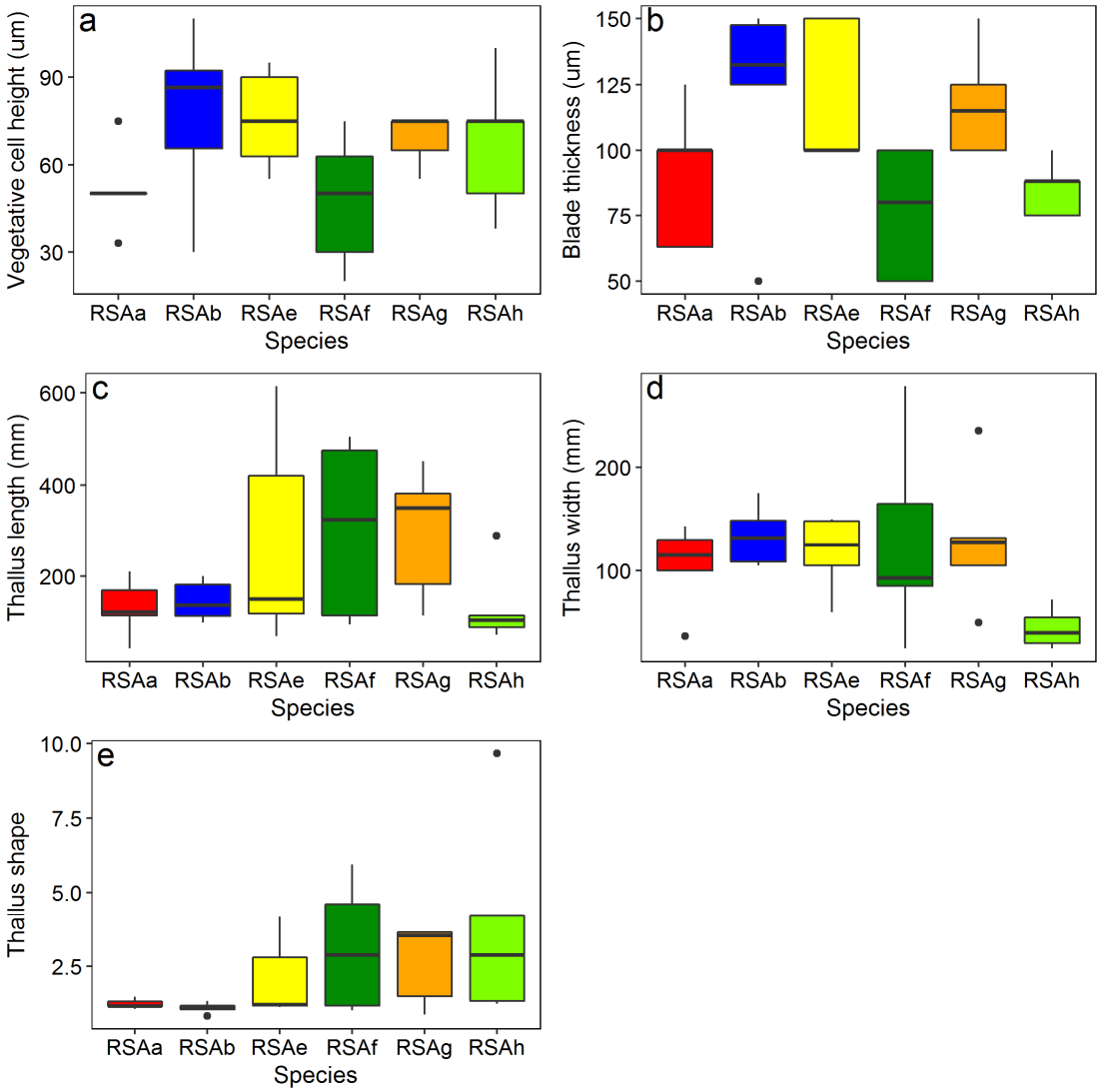

Fig. 4.3. Box and whisker plots showing a comparison of morpho-anatomical characters between molecular species that were represented by more than five specimens. Each molecular species is represented using a unique colour and label (on x-axis). The y-axis shows the median and range (95% confidence intervals) for each character; a) height of non-reproductive (vegetative) cells measured from a transverse section of the thallus; b) blade thickness measured from a transverse section of the thallus; c) length of thallus measured from the base of the plant to the apex; d) width of thallus measured from the widest points of the plant; e) shape of the thallus (L/W) which was used as an indication of overall shape (rosette or lanceolate).

Table 4.4. ANOVA results comparing morphometric and anatomical characters among molecular species (RSAa–h).

	Df	Sum Sq	Mean Sq	*F*-value	*P*
Non-reproductive cell size (µm)					
Species	5	4048	809.6	1.866	0.136
Residuals	25	10844	433.8		
Blade thickness (µm)					
Species	5	11098	2219.6	3.142	0.025*
Residuals	25	17659	706.4		
Thallus length (mm)					
Species	5	184720	36944	1.831	0.143
Residuals	25	504383	20175		
Thallus width (mm)					
Species	5	28981	5796	1.977	0.117
Residuals	25	73314	2933		
Thallus shape					
Species	5	30.91	6.181	1.899	0.130
Residuals	25	81.39	3.256		

* Significant

Ecological characters mostly overlapped between species as most species were collected between the low and mid-eulittoral zone and some extended further into the upper eulittoral (e.g. RSAf and RSAh). RSAh was confined to the upper eulittoral while RSAf was the most geographically widespread species and was found from the sublittoral to the upper eulittoral. A single specimen of RSAf was collected from Cape St. Francis, approximately 700 km east of other specimens included in this species. RSAf also included two records of species found subtidally: *Porphyra* sp. OD1 which was found growing on experimental aquaculture ropes deployed in Oudekraal at a depth of *ca*. 2–5 m (although it was attached closer to the surface) and *Porphyra* sp. SP1 which was found growing submerged in an experimental aquarium tank in Sea Point. RSAa was widespread throughout the eulittoral and a specimen collected from St. Helena Bay belonging to this species measured 1.2 m and is the largest specimen of *Porphyra* found in South Africa to date.

There was also no congruence between substratum (epilithic, epiphytic or epizoic) and different molecular species, and although there was some level of species dominance related to geographic location there was also co-occurrence of a few species at some sites, discussed below.

Geographic distribution of the **Porphyra capensis** *complex*

Reference/co-ordinates to sites mentioned can be found in Fig. 2.1 or Table 4.3. Species within the PCC were generally widely distributed along the west coast with many species occurring

sympatrically. RSAa was the most widely distributed species and occurred from Port Nolloth to De Kelders and may extend as far east as Knysna. However, it should be noted that no specimens have been found between De Kelders and Knysna. RSAe was the second most widely distributed species and occurred from Port Nolloth to Tsaarsbaai and was absent east of the Cape Peninsula. RSAb occurred from Kraalbaai to Suiderstrand (Cape Agulhas region) and may extend as far east as Plettenberg Bay. RSAf occurred from Yzerfontein to Kommetjie but may extend to Cape St Francis. RSAg occurs from Port Nolloth to Kleinzee and RSAh was only found in Yzerfontein. RSAd and c were represented by only one and two specimens and occurred in De Kelders and Strand, respectively. Species belonging to the PCC generally occurred sympatrically along its distribution range, and at localities on the Cape Peninsula as many as 4–5 species co-occurred on a single shore, although the occurrence of such a high number of species on a single shore was not common.

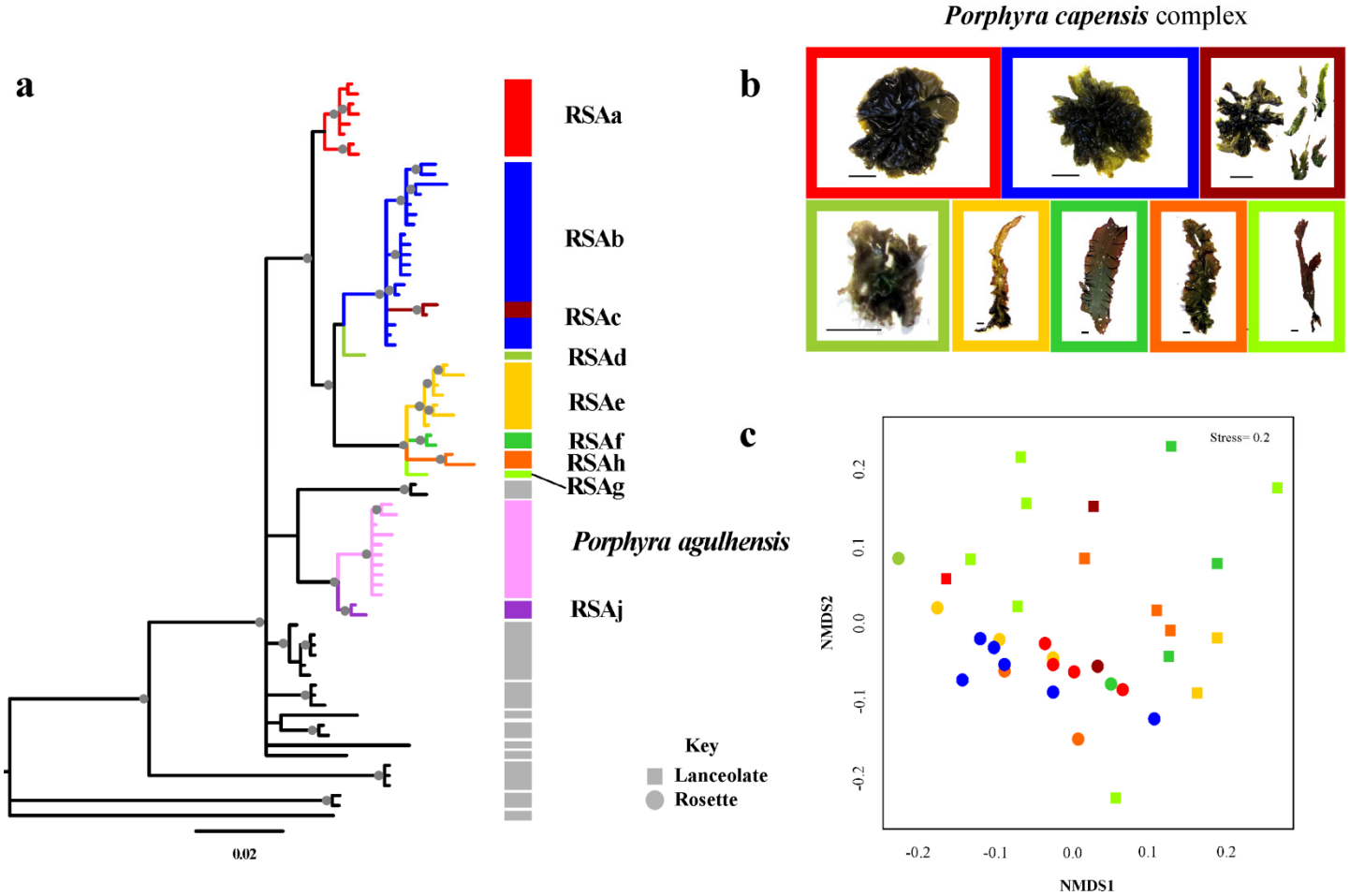

Fig. 4.4. An integrative species delimitation approach for identifying South Africa *Porphyra*. a) molecular phylogeny of *Porphyra* based on the *cox*1 gene, each molecular species is given a unique colour and label as in Chapter 2 and grey bars indicate species of *Porphyra* from elsewhere. Well-supported nodes are marked with a grey circle at the inter-node; b) morphological variation in the *Porphyra capensis* complex, borders are coloured according to molecular species as in (a); c) nMDS plot, points are coloured according to molecular species as in (a) and the shape of the points indicates overall morphology, rosettes (circles) and lanceolate (square).

***Key to the South African species of* Porphyra**

1a Blade thickness greater than 50 µm, 1–2 chloroplast(s) per cell, cells appear ovoid in cross section of the thallus, trichogynes absent.....*P. capensis* complex

1b Blade thickness less than 50(–75) µm, 1 chloroplast per cell, cells appear elliptical in cross section of the thallus, trichogynes present.....*P. agulhensis* duo

4.3.3. Phylogenetic relationships of South African Porphyra

The topology of the multigene phylogeny for *Porphyra* was retained as monophyletic and species boundaries confirmed for species from South Africa (according to Chapter 2) despite the addition of sequence data. Reference is therefore made to Fig. 2.2. A well-supported monophyletic clade contained all species of South African *Porphyra* with the exclusion of *Porphyra* ZSM and the inclusion of two molecular species from Chile (CHE & CHF). Within this clade two well-supported sub-clades were recovered, a clade with species predominantly present along the south-west and south coast of South Africa and another with species predominantly distributed along the west coast of South Africa and including the two Chilean molecular species.

Genetic diversity and distribution of genetic lineages of* Porphyra *in South Africa

Higher species diversity (8 out 10 species) was found on the west coast compared to the south coast (2 species) of South Africa and species from these respective coastlines did not overlap in distribution. Note that haplotype and nucleotide diversity takes into account varying population sizes and therefore indices are comparable between species. High genetic diversity with a high haplotype and nucleotide diversity for individual species within the *P. capensis* complex was found for both genes (*cox*1 & *rbc*L) (Table 4.5). The highly genetically variable *Porphyra capensis* complex (PCC) occurred mostly along the west coast of South Africa with the exception of a few specimens (*Porphyra* sp. CSF 2, KH1, PBB 3, 4, 6) collected on the south coast of South Africa. In contrast, *Porphyra agulhensis* and *Porphyra* RSAj were characterised by low genetic variation using both genes (Table 4.5). *Porphyra agulhensis* consisted of one major (82%) haplotype and nine private haplotypes for the *cox*1 gene. *Porphyra* RSAj was equally low in terms of its genetic composition and a single haplotype represented 88% of individuals in this species for the *cox*1 gene; however this was based on a relatively low sample size (n = 8).

Table 4.5. Diversity indices for South African *Porphyra* based on the *cox*1 and *rbc*L genes. N indicates the number of sequences, H is the number of haplotypes recovered, Hd the Haplotype diversity, Nd the nucleotide diversity, sd the standard deviation, and WC and SC denote the west and south coasts of South Africa.

Species	Locality	N *cox*1	N *rbc*L	H *cox*1	H *rbc*L	Hd (SD) *cox*1	Hd (SD) *rbc*L	Nd (SD<0.01) *cox*1	Nd (SD<0.01) *rbc*L	Variable sites *cox*1	Variable sites *rbc*L
P. capensis complex	WC	129	68	23	20	89 (0.01)	87 (0.03)	0.014	0.017	35	52
RSAa	WC	34	6	8	5	78 (0.05)	93 (0.12)	0.003	0.007	11	16
RSAb	WC	50	30	17	5	85 (<0.01)	53 (0.01)	0.006	0.005	25	28
RSAe	WC	26	11	7	6	69 (0.08)	73 (0.14)	0.002	0.003	10	10
RSAf	WC	7	4	2	3	48 (0.17)	82 (0.22)	0.001	0.001	1	4
RSAg	WC	5	N/A	2	N/A	40 (0.23)	N/A	0.005	N/A	4	N/A
P. agulhensis duo	SC	56	11	12	4	51 (0.08)	69 (0.13)	0.003	0.001	21	9
P. agulhensis RSAj	SC	48	9	10	3	34 (0.09)	56 (0.16)	0.001	0.000	13	2
	SC	8	N/A	2	N/A	25 (0.18)	N/A	0.001	N/A	2	N/A

Haplotype networks were used to illustrate the genetic diversity and distribution of genetic lineages within and among cryptic species groups of *Porphyra* along the South African coast based on the *cox*1 gene (Fig. 4.4).

Within the PCC, individual species were generally widely distributed throughout the west coast with some species more abundant than others in some Zones (Fig. 4.4 & Fig. 4.5). For example, RSAa occurred across three upwelling zones, Zones 1–3, and three non-upwelling zones, Zones, FB, EOCH and SC. This species was most common in Zones 1 and 2 and consisted of two dominant and several private haplotypes. The larger of the two haplotypes was restricted to Zone 1 and the other occurred along Zone 2 and 3 with many smaller groups or private haplotypes along Zone 3. RSAe was the second most widely distributed species and spanned across upwelling Zones 1–3. This species was most abundant in Zone 3 and absent east of the Cape Peninsula. RSAe consisted of two major haplotypes; the larger of the two was restricted to Zone 2 and the much smaller one found along Zone 1. RSAb occurred mostly along two upwelling zones, Zone 3 and 4, and two non-upwelling Zones, FB and EOCH (to Cape Agulhas), and may extend as far east as Zone SC. This species was most abundant in Zone 3 and was absent further north of this Zone. Two major haplotypes were present and these occurred on either side of Cape Point, a major biogeographic barrier along the South African coast. All other species appear to be geographically restricted: RSAf and RSAh were confined to upwelling Zone 3, and RSAg was confined to the upwelling Zone 1. RSAd and c were represented by only one and two specimens, respectively, and occurred in two-non upwelling Zones, EOCH and FB, respectively. *Porphyra agulhensis* and *Porphyra* RSAj both occurred in non-upwelling Zones, SC and SEC.

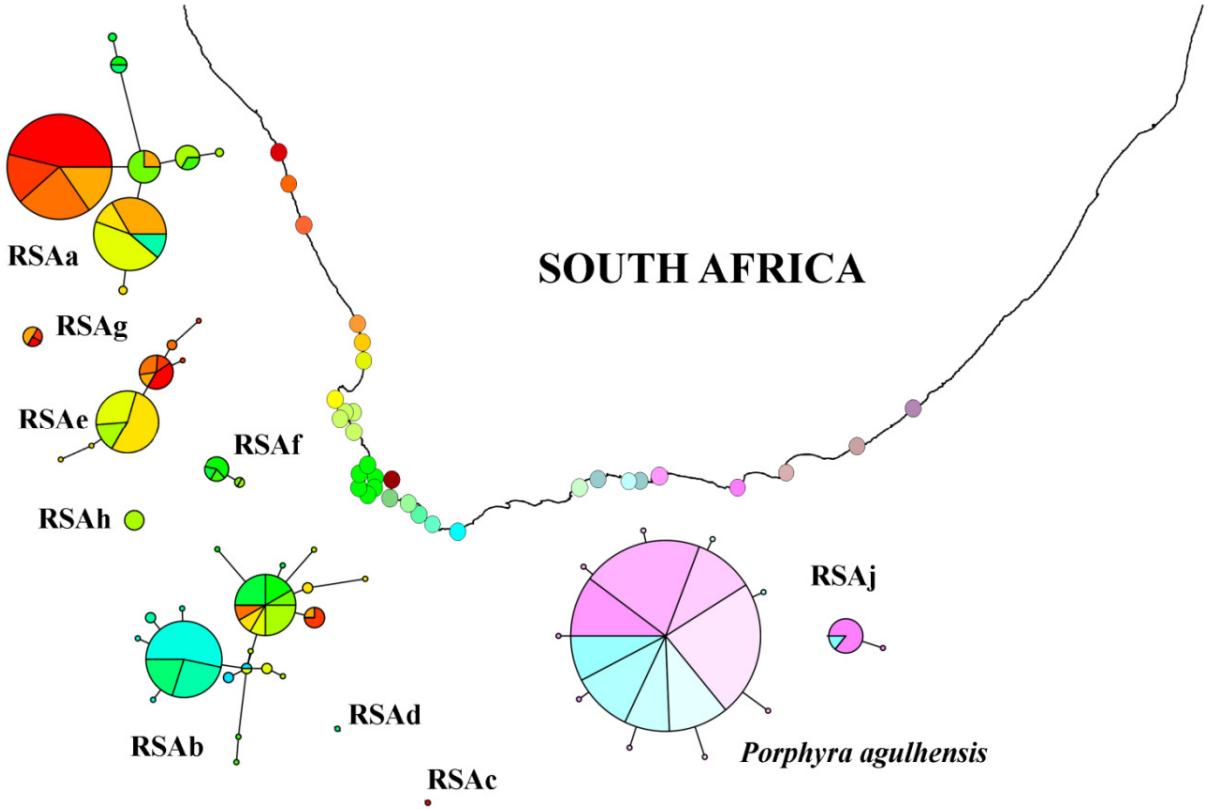

Fig. 4.5. Haplotype network based on the *cox*1 gene showing the genetic variation within species and relatedness amongst species in relation to geographic distribution. Each species is represented individually as a pie chart, slices of the pie are coloured by site and the size of the pie is proportional to the number of individuals included in a particular haplotype. Solid lines indicate intraspecies variation.

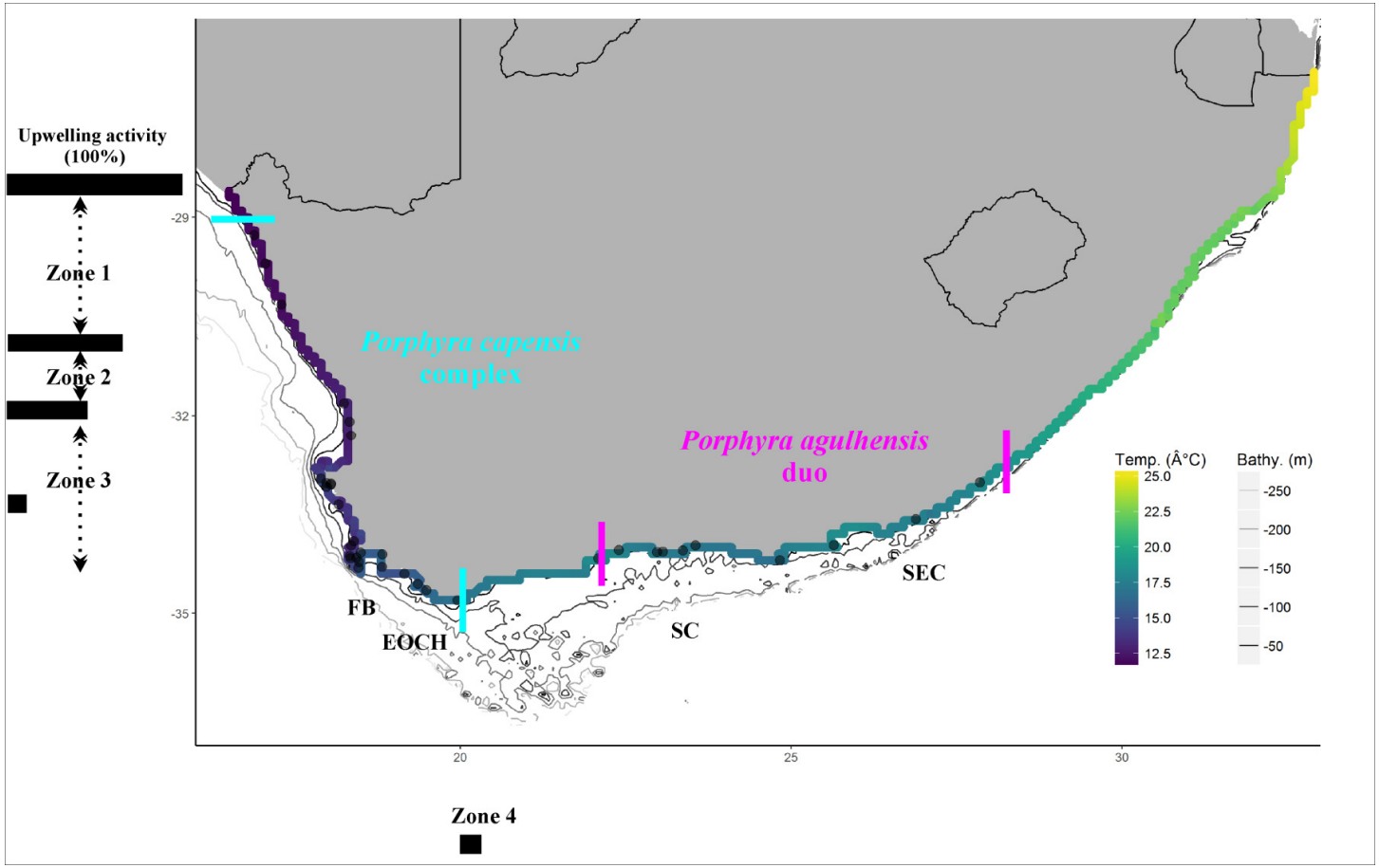

Fig. 4.6. Patterns of diversity in South African *Porphyra* related to differences in coastal features such as temperature, upwelling regions and bathmetry. The distribution range of the species groups are demarcated with solid lines (blue for the *Porphyra capensis* complex and pink for the *Porphyra agulhensis* duo) . Sample sites are indicated with small black circles along the coastline. Upwelling zones and activity and non-upwelling zones FB = False Bay, EOCH = East of Cape Hangklip, SC =South coast and SEC = South-east coast were redrawn from the literature. Bathymetry and *in-situ* temperature along the coastline demarcated and coloured according to key.

Comparative phylogeography and demographic history of the cryptic species in the* Porphyra capensis *complex and* P. agulhensis *duo along the South African coast

Specimens of *Porphyra* were collected from the South African coast at random and sorted into cryptic species only after sequencing various genes. This meant that species contributed vastly different numbers of specimens, eg. RSAb had 50 specimens and RSAc contained 2. The four species that were selected for phylogeographic analyses were those containing an adequate number of samples for comparisons. Three species from the PCC, namely RSAa, RSAb and RSAe, and one species from the *P. agulhensis* duo were analyzed.

Comparative phylogeography and demographic history

Phylogeographic structure in individual cryptic species was found in the PCC for species RSAa, RSAb and RSAe, but no genetic structure was found in *P. agulhensis*. The widespread RSAa was the only species to occur across three upwelling zones and 80% of the genetic variation in this species was partitioned across these zones (Table 4.5). Strong genetic breaks were observed between upwelling Zone 1 and Zone 2, as well as between Zone 1 and Zone 3 for cryptic species RSAa (Fig. 4.6). For this species, individuals in False Bay were also highly differentiated from all other sites. Strong genetic structure between Zone 1 and Zone 3 was also found in cryptic species RSAe (Fig. 4.6). Lastly, a strong genetic break was evident between Zone 3 and Zone 4 in cryptic species RSAb (Fig. 4.6).

Moderate genetic structure was found in cryptic species RSAa and RSAb between Zone 2 and Zone 3 and between EOCH and False Bay, respectively (Fig. 4.6) On the contrary, along the south coast of South Africa, geographic populations of *P. agulhensis* were panmictic (no genetic structure) throughout its distribution range (Table 4.6).

The only inference that could be made about the demographic history of species concerned RSAj, in which Tajima's D and Fu's Fs suggest a recent selective sweep or expansion after a population bottleneck (Table S6). Mismatched distribution plots suggest that species within the PCC indicate a demographic or range expansion (Fig. S5–8).

Table 4.6. AMOVA comparisons for genetic partitioning amongst zones for four species of *Porphyra* based on the *cox*1 gene. Df indicates the degrees of freedom, SS indicates the sum of squares and PhiPT denotes the level of genetic structure.

Hypothesis	**Source of variation**	***Df***	**SS**	**Variance components**	**Variance (%)**	**PhiPT**	***P***
RSAa	Among sites	3	26.413	8.804	80%		
	Within sites	29	8.071	0.278	20%		
	Total	32	34.485		100%	0.798	0.010*
RSAb	Among sites	3	38.378	12.793	47%		
	Within sites	43	49.324	1.147	53%		
	Total	46	87.702		100%	0.467	0.001*
RSAe	Among sites	1	5.844	5.844	76%		
	Within sites	21	3.373	0.161	24%		
	Total	22	9.217		100%	0.764	0.010*
P. agulhensis	Among sites	1	0.355	0.355	1%		
	Within sites	46	13.311	0.289	99%		
	Total	47	13.667		100%	0.009	0.030*

*= denotes significant

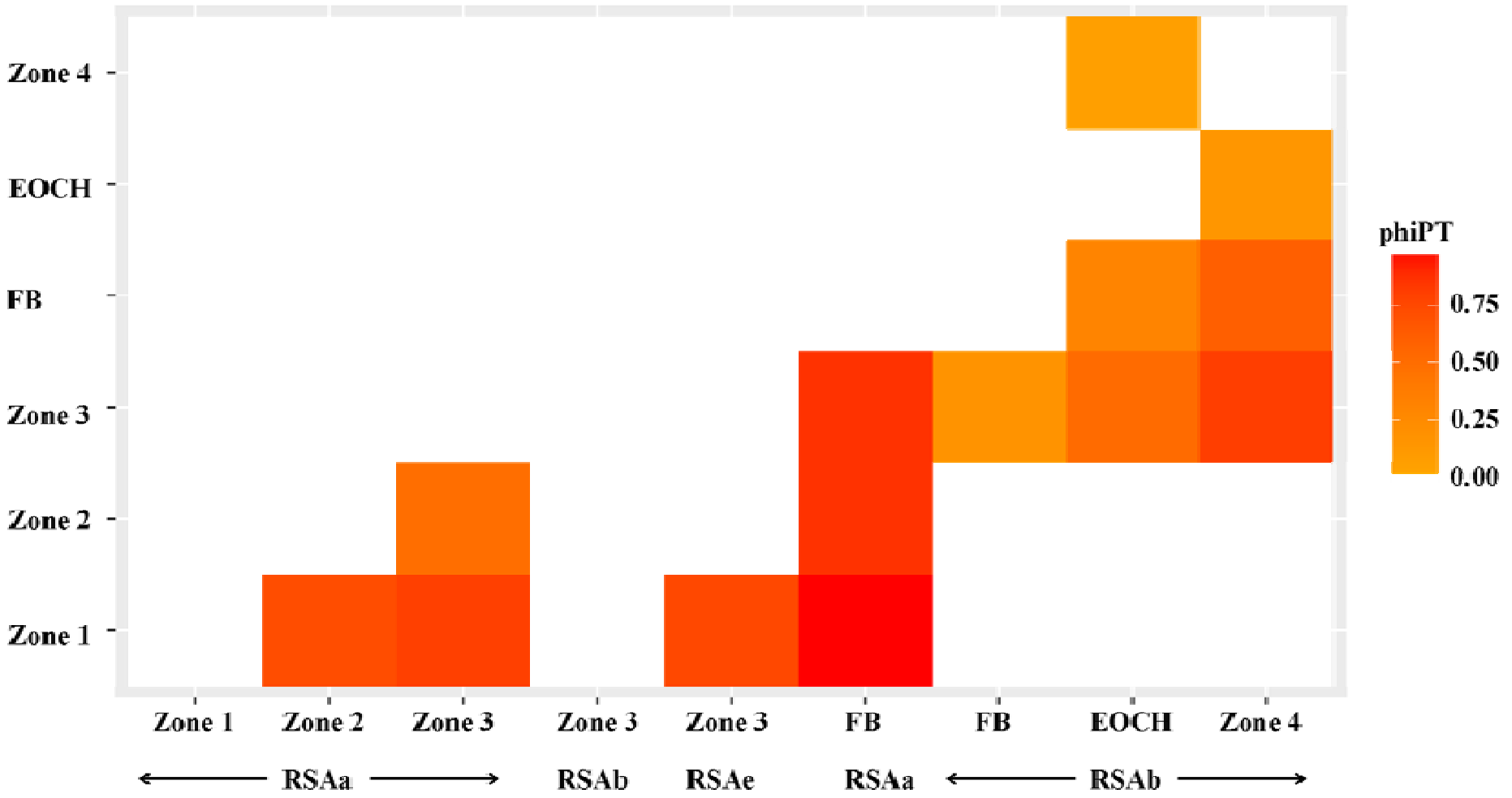

Fig. 4.7. Heat map showing pairwise phiPT comparisons between zones and species based on the *cox*1 gene. Zones 1–4 demarcate regions of the coastline as in Fig. 4.5 and RSAa, b and e indicate molecular species in each of the Zones.

4.4. DISCUSSION

The morphological species '*P. capensis*' was shown to comprise multiple cryptic species including one newly described species, *Porphyra agulhensis*, based on an integrative taxonomic approach (molecular, morphological & ecological data). All species of *Porphyra* from South African form a single monophyletic group based on a multigene phylogeny and can be regarded as two morphologically distinct species groups, each occupying different biogeographic regions. The *P. capensis* cryptic species complex (PCC) occurs along the cool temperate Atlantic coastline on the west coast (Benguela region) of South Africa, and the newly described *Porphyra agulhensis* duo occurs along the warm temperate Indian Ocean coastline on the south coast (Agulhas region) of South Africa. This suggests that the Benguela/Agulhas transition zone may have acted as a strong genetic barrier to gene flow in the genus *Porphyra* in South Africa. Early evidence for upwelling-driven phylogeographic (intraspecific) structure in some cryptic species was also found and warrants further investigation.

These results compare well with other examples of species that were previously considered single and widespread taxa in South Africa but were found to include multiple species or cryptic species. For example, the broadcast spawning African crown crab is widely distributed along the southern African coast, where it is endemic, and was found to consist of multiple species including cryptic species (Edkins *et al*., 2007). Similarly, the broadcast-spawning ascidian, *Pyura stolonifera* (Teske *et al*., 2011b) and two live-bearing and endemic South African clinid fishes have also been found to comprise cryptic species (von der Heyden *et al*., 2011). In the latter case different cryptic species were found to occupy different biogeographic regions, which is similar to cryptic species groups of *Porphyra* in South Africa.

4.4.1. The **Porphyra agulhensis** *species duo*

The *Porphyra agulhensis* duo fits the description and distribution range of the dwarf form of '*P. capensis*' documented by Isaac (1957) and Graves (1969). *Porphyra agulhensis* was identified as being a distinct molecular entity (ZPP) based on a nSSU phylogeny (Jones *et al*., 2004) and was later supported as being distinct using *rbc*L sequence data (Sutherland *et al*., 2011). However, neither study made the association between ZPP and the dwarf *P. capensis* form. More recently, a comprehensive biodiversity assessment of the Bangiales along the South African coast identified a distinct molecular species, RSAi, corresponding to the entity ZPP (Chapter 2). In the present study, distinct morpho-anatomical and ecological traits congruent with the molecular species delimitation of RSAi (Chapter 2) were found. The molecular entities ZPP and RSAi, as well as the dwarf form of '*P. capensis*', all of which represent a single species, is formally named here as *P. agulhensis*.

Porphyra agulhensis is characterized by its diminutive size, delicate laciniate rosette blades, and distinct greenish to pale pinkish-purple colour. It is easily distinguished from the PCC by its overall morphology, the presence of distinct trichogynes, intermingled spetmatangial and zygotosporangial sori and its poor adherence to herbarium paper. All species of the PCC adhere comparatively well to herbarium paper. Herbarium records corresponding to the characteristic appearance of *P. agulhensis* were added to our study. Such herbarium records are invaluable when defining the distribution limits and seasonal occurrence of species (Brodie *et al*., 2008b; Nelson *et al*., 2013). Combined results (new collections & herbarium records) from the present study indicate that the *P. agulhensis* duo occurs throughout the year along the south coast of South Africa where it is endemic. Many other species of seaweed are also endemic to this region (Stegenga *et al*., 1997; Anderson *et al*., 2009). Species from the North Atlantic, such as *P. umbilicalis* (Conway, 1964), *P. dioica* (Brodie & Irvine, 2003) and *P. purpurea* (in Holmes & Brodie, 2004) are also known to occur year-round. On the other hand some species of *Porphyra* in New Zealand were found to occur for only a few months of the year (Schweikert *et al*., 2012).

P. agulhensis occurs in a unique region along the South African coast bordered by western cooling and eastern warming and its distribution may be limited by physiological constraints. A second species RSAj, which has an overlapping geographical range and similar morphology to *P. agulhensis* is currently regarded as a cryptic species. This cryptic species was not described based on fixed nucleotide differences because it was represented by a relatively small sample size and was recorded in only two sites. However further research on this cryptic species may yield more data and distinguishing features that may allow for the description of this species based on fixed nucleotide differences. *Porphyra agulhensis* is commonly found in the mid-eulittoral to upper eulittoral in small, isolated patches in the winter and larger, denser patches in the summer. This species is generally much less abundant than the *P. capensis* complex on the west coast of South Africa (Maggie Reddy personal observation 2014–2018). Its abundance and related distribution follows a typical central-abundance pattern (around Port Elizabeth). This phenomenon (central-abundance pattern) has also been found in some species of brown algae (Veijo *et al*., 2011; Zardi *et al*., 2015; also see Sagarin & Gaines, 2002). However, further sampling over different seasons and over a longer time is required to confirm this pattern.

4.4.2. The **Porphyra capensis** *cryptic species complex*

For the remaining eight molecular species of *Porphyra* (RSAa–h) results consistently indicated that no single diagnostic character (morpho-anatomical and/ ecological features) or combination of characters could be used to reliably distinguish between species. Blade thickness was shown to be significantly different between some species. However, although blade thickness differed between

some species, this character generally overlapped with other some other species and was therefore not diagnostic. Since the type specimen was unavailable and an online examination provided no additional information, RSAa–h are therefore only detectable by sequence data and must be regarded as cryptic species, unless further investigations show otherwise. This phenomenon would explain why Isaac (1957) and Graves (1969) regarded them as a single morphologically variable species. Cryptic diversity is common in the bladed Bangiales and has been reported in various parts of the world (Stiller & Waaland 1993; Brodie *et al*., 2008a; Lindstrom, 2008; Niwa *et al*., 2014), as well as for other seaweeds (Zuccarello & West, 2003; Saunders 2008; Kraft *et al*., 2010; Payo *et al*., 2013; Muangmai *et al*., 2014; Montecinos *et al*., 2017a) and for other marine taxa (Knowlton, 1993).

Cryptic species along the west coast of South Africa (RSAa–h) were collectively referred to as the *P. capensis* complex for the following reasons. Firstly, morpho-anatomical characters of these cryptic species (RSAa–h) were within the range of the current description of *P. capensis*. Secondly, although the exact type locality of *P. capensis* is unknown (see Chapter 2 for details), the current description and appearance of *P. agulhensis* excludes the south coast region as a possible locality. It is therefore almost certain that the type locality of *P. capensis* lies on the west coast of South Africa. Lastly, due to a lack of sequence data from the type specimen, the name cannot be attached to a single species and the *Porphyra capensis* cryptic species complex was therefore adopted to collectively refer to all cryptic species of *Porphyra* from the west coast of South Africa.

The PCC was largely restricted to the west coast of South Africa, but a low incidence of specimens (<4% of plants) were found at three sites along the south coast (as far east as Cape St. Francis) during the winter. This appears to be unusual because there is no herbarium records in BOL of the PCC collected from the south coast of South Africa. However, examination of specimens from GRA (which is the largest seaweed collection from the south coast of South Africa) is required to confirm this.

The general morphology of *Porphyra* (rosette or lanceolate) has been considered a taxonomically informative character. However, in many instances in the PCC, while up to 85% of individuals presented one form, some individuals presented the other form. Such morphological plasticity suggests that this trait has not been completely fixed in the PCC, therefore limiting our ability to refer to individual species as being strictly rosette or lanceolate. These results are consistent with shore experiments by Isaac (1957), in which rosette forms of '*P. capensis*' commonly inhabiting the sublittoral were relocated higher on the shore and over time exhibited a more lanceolate appearance. This suggests that a change from a rosette form to a linear one is not phylogenetically informative for the bladed Bangiales and may rather be an ecophysiological response (Isaacs, 1957; Kavale *et al*., 2015) or a response to seasonal variability (Holmes & Brodie, 2004).

For a species that tends to have a rosette shape, mechanical processes such as intense wave action, grazing and weathering may account for a lanceolate appearance. However, the occasional rosette form found in a species with predominantly lanceolate specimens is more difficult to explain, and may be a response to desiccation. To further complicate the apparent switch between a rosette and lanceolate form, both forms can co-occur at a single location at the same shore position and individuals of the same species (e.g. RSAe) can appear lanceolate (e.g. OD1) on the west coast and rosette (e.g. CSF2) on the south coast. This implies that ecophysiology may not fully explain a rosette or lanceolate appearance. Alternatively, general form may be governed by both a genetic and ecological component. Another consideration is that genes used in the present study are not likely to be directly associated with the morphological appearance of these algae and a more targeted genomic approach (Brawley *et al*., 2017) might better explain trait variation in the morphologically simple but variable PCC.

Incongruence between morphological and molecular trait variation has also been found in species of *Porphyra* in New Zealand and the North Atlantic. In New Zealand, species of *Porphyra* are known to exhibit a high degree of morphological variation, but this variation does not align with genetic variation in the nSSU gene (Broom *et al*., 1999). In the same way, morphological differences in *P. purpurea* in the North Atlantic were found to be inconsistent with genetic differences in the *rbc*L and ITS genes (Bray *et al*., 2006). However, differences in reproductive strategy (monoecy or dioecy, respectively) in *P. purpurea* and *P. dioica* were found to align with genetic differences in the *rbc*L spacer region (Brodie *et al*., 1996). This suggests that particular genes may be associated with certain gene regions.

Species of the Bangiales have been shown to vary ecophysiologically, particularly in their stress tolerance, which thus determines their position on the shore (Nelson *et al*., 2005; Bödeker *et al*., 2008; Schweikert *et al*., 2010). Studies have shown that different cryptic species of certain brown algae tend to occupy different shore positions (Montecinos *et al*., 2017a; Neiva *et al*., 2017). Additionally, some species of the bladed Bangiales in Japan (Miyata & Kikuchi, 1997) and South Africa (Griffin *et al*., 1999a) are known to differ in their substratum preference. However, in the present study no obvious pattern was found in the distribution of cryptic species in the PCC in relation to shore position or substratum. This is not uncommon in the bladed Bangiales as other closely related species are known to occur sympatrically, in other parts of the world (Schweikert *et al*., 2012; Niwa *et al*., 2014; Guillemin *et al*., 2016). Ecophysiological differences in the conchocelis phase may contribute to the co-occurrence and geographic distribution of species of the Bangiales (Clokie & Boney, 1980; Waaland *et al*., 1990). Other mechanisms that may facilitate the co-occurrence of cryptic species at the same geographic locality and at the same shore position include reproductive barriers such as pre- or-post meiotic barriers (Hoarau *et al*., 2015; Montecinos *et al*., 2017b) or non-overlapping temporal

reproductive cycles (Monteiro *et al*., 2016) which allow species in other algal groups to occur sympatrically.

Due to the lack of morphologically distinguishing characters in the PCC, herbarium records could not be used to determine the seasonality and distributional limits of individual species in this complex, and further research is required regarding these aspects. Nevertheless, the *P. capensis* complex occurs year-round and maintains high standing stocks, particularly in the winter months around the Cape Peninsula (Griffin *et al*., 1999b), and is a dominant component of the intertidal on both sheltered and wave exposed shorelines along western South Africa.

4.4.3. Genetic diversity of the genus **Porphyra** *in South Africa in relation to biogeography*

The *P. agulhensis* cryptic species duo along the warm temperate south coast of South Africa was characterized by very low genetic diversity and no evidence of regional sub-structure within each species. Such low levels of genetic variation can indicate a recent colonization (Maggs *et al*., 2008; Chan *et al*., 2014) or a recent bottleneck and/or inbreeding (Nei *et al*., 1975; Palkopoulou *et al*., 2015; Almedia *et al*., 2017; but see Assis *et al*., 2013).

In contrast, the PCC along the cool temperate Atlantic shoreline of the west coast of South Africa is genetically diverse and rich in species. The distribution of the PCC followed a general pattern of incomplete species dominance related to geographic areas (Zones). This pattern could indicate semi-permeable genetic barriers (discussed below) or retention of ancestral polymorphism, the latter of which may be common if a large effective population size is maintained through time (Charlesworth, 2009). However, despite this general pattern, some species occur sympatrically throughout the distribution range of this complex. High levels of genetic diversity along the west coast of South Africa have been found in other temperate-adapted organisms, such as species of goby (von der Heyden *et al*., 2011) and in the widely distributed barnacle, *Tetraclita serrata* (Reynolds *et al*., 2014). However, in a study of various marine organisms exhibiting different life history characteristics, low genetic diversity was found on the west coast compared to the south and east coasts of South Africa (Wright *et al*., 2015). Likewise, a more recent study on seaweeds showed that a gradient in β-diversity (based on morphological identification) occurs along the south and east coasts of South Africa, but not along the west coast (Smit *et al*., 2017). However, the former study did not take into account whether organisms were temperate or tropical in affinity and the latter study did not account for cryptic species, which may conceal species turnover.

Two possible scenarios may explain patterns of speciation in *Porphyra* as related to inshore marine biogeography in South Africa. Past isolation could have driven speciation and subsequent present day environmental differences in each region (especially seawater temperature and oceanographic

conditions governing dispersal) might maintain the isolation of genetic lineages or closely related species on either side of this transition zone, as has been proposed for other marine organisms along this coastline (von der Heyden *et al*., 2009; Teske *et al*., 2011a). Alternatively, two species radiations may have occurred along the South African coast. In this case *Porphyra* may have originated and diversified along the south-west coast of South Africa, where high levels of genetic diversity have been found, and then later a single clade (*P. agulhensis* & RSAj) may have dispersed to the south coast and another along the west coast of South Africa. For the south coast clade this could have occurred in response to more favourable environmental conditions (see later).

A shared evolutionary history for some species of *Porphyra* from Chile and South Africa provides further support for the proposal by Hommersand (1986) of past connectivity in the Southern Ocean for red algae. However, the close phylogenetic affinities between these species suggest a more recent dispersal event than that proposed by Hommersand (1986) and for species of South African *Pyropia* (Chapter 3).

4.4.4. Could upwelling be driving intraspecific phylogeographic structure and speciation in the **Porphyra capensis** *complex in the southern Benguela?*

Unlike the large discontinuity in abiotic factors such as currents, nutrients and temperature that exist between the Atlantic (Benguela region) and Indian (Agulhas region) Oceans and that may explain patterns of speciation in the different cryptic species groups of *Porphyra*, no obvious abiotic gradients exists along the cool temperate west coast of South Africa (Fig. 5; however see Rothman *et al*., 2017 regarding a turbidity gradient offshore). This region, however, is highly influenced by the Benguela Current and various upwelling cells which create a highly variable environment (Isaac 1937; Shannon 1985). Prevailing ocean currents and upwelling have been shown to act as vicariance barriers to gene flow for a broad-cast spawning New Zealand sea star (Waters & Roy, 2004), a New Zealand chiton (Veale & Lavery, 2011), the South African Cape sea urchin (Muller *et al*., 2012) and the cosmopolitan bluefish (Reid *et al*., 2016).

In the present study the level of phylogeographic structure or speciation found in various cryptic species of *Porphyra* could be related to the distribution, intensity and frequency of upwelling cells. Regions with higher upwelling intensity coincided with more genetically structured lineages compared to regions with lower upwelling intensity. Upwelling cells (Zones 1–4) decrease in upwelling intensity from north to south along the west coast of South Africa and correlated with lower genetic differentiation within species of the PCC (intraspecific variation). The highest level of genetic structure occurred between Zone 1 and 2, where more intense upwelling occurs, while the lowest genetic structure occurred in Zone 3 and east of Zone 3, where weak upwelling occurs. Along the south coast of South Africa, where upwelling is much less frequent and less intense, *P. agulhensis*

showed little to no phylogeographic structure. A lack of phylogeographic structure and asymmetric gene flow along the south coast has been shown for other marine organisms (Teske *et al*., 2011a). However, it is worth noting that the lack of phylogeographic structure in *P. agulhensis* could be a result of a recent colonization event (discussed earlier).

Upwelling in the Benguela region dates back 12 MYA, and oscillated between periods of stronger and weaker upwelling (and possibly no upwelling), but the existing regime has remained relatively stable in the last 3–4 MY (Shannon, 1986; Marlow *et al*., 2000). Therefore, it is also tempting to speculate that the high rates of speciation and species richness along the west coast of South Africa might be a consequence of historic and persistent upwelling events, that may have at various times created phylogeographic breaks that led to speciation. Alternatively, climatic oscillations, dispersal or biotic limitations could have been involved. Climatic oscillations during cycles of glacial maxima and minima caused changes in sea levels, suitable habitat and other environmental conditions, and could have fragmented the continuous distribution of a single species (Toms *et al*., 2014). Under such conditions, fragmented populations that were isolated in multiple local refugia could have diverged and subsequently re-colonized previous habitats during more favourable conditions (Li *et al*., 2016). This could explain genetic patterns in *Porphyra* in South Africa and the co-occurrence of various species within a common area. The high genetic diversity along Zone 3 indicates that this region may have been an ancestral refuge that has since maintained much of that diversity. Upwelling regions have recently been shown to act as cold-water climate refugia for temperate seaweeds in the North Atlantic (Lourenco *et al*., 2016) and this could have similarly occurred for South African *Porphyra*.

4.5. CONCLUSION

Species boundaries for molecular species identified in Chapter 2 were further tested using morph-anatomical characters, ecological features and additional sequence data. This integrative approach resulted in the recognition of multiple cryptic species on the west coast of South Africa and these were collectively referred to as the *P. capensis* complex. New collections supplemented with herbarium records showed that *Porphyra* along the south coast of South Africa has been erroneously identified as *P. capensis*, and represents a new species here named *P. agulhensis*. A cryptic species that is molecularly distinct but morphologically similar to *P. agulhensis* is also recognized. This species duo is most abundant during the summer and its distribution is restricted to the Agulhas Marine Province (south coast of South Africa). These results demonstrate the need for the re-examination of widely distributed or morphologically variably species using an integrative approach which includes molecular data. It is hypothesized that South African *Porphyra* originated and diversified along the south-west coast. At least two species radiations have occurred along this coastline; one that included species on the south coast of South Africa and a second radiation along

the west coast of South Africa. Evidence for past connectivity in the Southern Ocean is further supported because South African species of *Porphyra* share a close phylogenetic relationship with two species from Chile. In South Africa, patterns of genetic diversity among species were related to biogeography (notably in seawater temperature), with higher levels of diversity and species richness on the west coast compared to the south coast of South Africa and a region of major biogeographic change between Cape Agulhas and Cape Point. Phylogeographic structure within cryptic species (intraspecific variation) was related to upwelling along the west coast of South Africa which is hypothesized to have been a major genetic barrier and similar upwelling-driven historic events could explain high rates of speciation in *Porphyra* in South Africa.

CHAPTER 5

General Discussion

In the first chapter a comprehensive biodiversity assessment was carried out, based on an extensive collection of specimens from the entire known distribution range of the Bangiales, along the South African coast. The application of DNA-based species delimitation methods including ABGD, GYMC and PTP, applied to the *cox*1 and *rbc*L genes and monophyly inferred from a multigene phylogeny resulted in the recognition of 10 *Porphyra* and three *Pyropia* species in South Africa. Only three of which had been previously described, (*P. capensis*, *Py. saldanhae* & *Py. aeodis*). Additional species of Bangiales previously recorded along the South African coast were added to the final species list despite not being found in the present study. This confirmed that three genera, the filamentous *Bangia*, and bladed *Porphyra* and *Pyropia* were present along this coastline and resulted in the recognition of an estimate of 14–16 species (Table 5.1). All species confirmed using genetic data, are likely to be endemic to southern Africa, but critical comparisons are needed for much of the Southern Ocean. Nevertheless these results compare well with other Southern Hemisphere countries, such as Chile (Guillemin *et al*., 2016) and New Zealand (Broom *et al.*, 1999, 2004; Nelson et al., 2001; Nelson, 2013) where high genetic diversity, species richness and endemicity have also been found.

In recent years there has been a general trend toward the application of analytical algorithms for delimiting species, based on multiple gene regions amplified from an extensive collection of specimens covering the entire distribution range of a species (Leliaert *et al*., 20104). This approach is intended to reduce the subjectivity that can be associated with defining species boundaries from monophyly alone or based on a single gene. DNA-based species delimitation works well for species groups with limited morphological features for identification. As such these algorithms have been recently applied to the bladed Bangiales in Chile (Guillemin *et al*., 2016), China (Yang *et al*., 2017b) and South Africa (Chapter 2). These studies show promise for the application of such methods in future taxonomic studies of the bladed Bangiales.

Species defined in Chapter 2 were delimited in the context of known genetic differences between species in each genus. This differed for *Porphyra* and *Pyropia* (3X higher) and therefore each genus was analyzed separately. Species boundaries for South African taxa delimited in the present study were therefore largely dependent on previous species concepts of *Porphyra* (Jones *et al*., 2004; Sutherland *et al*., 2011) and *Pyropia* (Stegenga *et al*., 1997; Griffin *et al*., 1999a). *Porphyra purpurea*, *P. dioica* and *P. corallicola* were recovered as sister clades to all other species of *Porphyra* (Supplementary S1) and genetic differences for the *cox*1 gene were similar to genetic differences between different genera in this order (an average of 13–15% & as high as 17%). The same was true for the first two species for the *rbc*L gene (Supplementary S2). This supports the prediction in Chapter 1 that the genus *Porphyra* may be further divided in future studies.

Table 5.1. Summary of Bangiales in South Africa.

Name	Functional form	Reproductive state	Colour	Substratum	Shore position	Distribution	Notes
Bangia fuscopurpurea (as *B. atropurpurea*)	Filamentous	Unknown	Dark red to black	Epilithic	Upper intertidal and supratidal	Dalebrook, Two Oceans aquarium and Langebaan Lagoon in South Africa; Cosmopolitan, found in estuaries and freshwater	Uncertain taxonomic identification
Porphyra capensis complex	Bladed, monostromatic	Monoecious and dioecious	Olive green to dark blackish-purple	Epilithic and Epzooic	Low eulittoral to upper eulittoral	Commonly on the west coast of South Africa	Currently regarded as a cryptic species complex
Porphyra RSAa							Cryptic species
Porphyra RSAb							Cryptic species
Porphyra RSAc							Cryptic species
Porphyra RSAd							Cryptic species
Porphyra RSAe							Cryptic species
Porphyra RSAf							Cryptic species
Porphyra RSAg							Cryptic species
Porphyra RSAh							Cryptic species
Porphyra ZSM							Identified using only molecular data
Porphyra agulhensis	Bladed, monostromatic	Monoecious	Olive green to pale purple	Epilithic and epiphytic	Low eulittoral to upper eulittoral	Restricted to the south coast of South Africa	New species, (previously misidentified as *P. capensis*)
Porphyra RSAj							Cryptic species
Pyropia aeodis	Bladed, monostromic	Monoecious	Red-brown to olive green	Epiphytic (*Aeodes orbitosa*)	Mid to low eulittoral	West coast of South Africa, possibly extending to northern	Species boundaries confirmed

Pyropia saldanae	Bladed, monostromic	Monoecious	Maroon to Dark purple	Epilithic	Lower eulittoral to sublittoral	Namibia Rooiels to Hondeklipbaai, possibly extending into Namibia	Species boundaries confirmed
Pyropia meridionalis	Bladed, monostromic	Monoecious	Dark purple to pink	Epizootic on *Cymbula compressor* or epiphytic on kelp	Subtidal	Soetwater to Klienzee	New species, (previously misidentified as *Py. gardneri* in South Africa)
Pyropia cf. *suborbiculata*	Bladed, monostromic	Unconfirmed	Pale pink	Epilithic	Low eulittoral	Cape Infanta, south coast of South Africa; Cosmopolitan	Uncertain taxonomic identification

For the present study, three routinely applied genes were selected (mitochondrial, *cox*1; chloroplastic, *rbc*L & nuclear, nSSU) and allowed for differences to be captured at varying resolutions. The selection of genes for DNA-based species delimitation was based on the most commonly collected Bangiales in South Africa (i.e. *Porphyra*). That meant that there was a bias toward the bladed Bangiales, with many more specimens of *Porphyra* than *Pyropia*. No new collections of any filamentous Bangiales were found despite several dedicated field surveys and therefore this study only included a few herbarium specimens of the filamentous Bangiales which could not be sequenced. As mentioned earlier the genus *Porphyra* is characterised by low genetic divergence with recently diverging species and for this reason DNA-based species algorithms were applied to the fast evolving, *cox*1 and *rbc*L, as these genes were more likely to capture species-level differences. However, one of the caveats of using the *cox*1 gene for South African Bangiales was the non-amplification for some specimens using published primers. DNA degradation was excluded as a probable cause as specimens that could not be amplified for the *cox*1 gene could be amplified for the *rbc*L gene. A further literature review indicated that introns may be present in this gene region for other Bangilean species. As such new primers were developed for presumed intron-containing specimens and proved to work.

The selection of the *cox*1 and *rbc*L genes, have been used for DNA-based species delimitation for Chilean bladed Bangiales (Guillemin *et al*., 2016). These markers may have similarly been employed in order to capture difference between closely related or recently diverging taxa (e.g. differences within *Porphyra*). However, in a study of Chinese bladed Bangiales, the *rbc*L and conserved nSSU genes were used for DNA-based species delimitation (Yang *et al*., 2017b). The selection of more conserved genes in this study may have been for direct comparison with Sutherland *et al*. (2011). Incidentally only *Pyropia* was found to be present in this region and the selection of genes were therefore suitable to capture species level differences. Globally, species of *Pyropia* appear to be much more divergent than species of *Porphyra*. In future studies, especially in regions where the Bangiales have not yet been studied using a molecular approach, a first step should be a preliminary biodiversity assessment using the *rbc*L gene followed by careful selection of a second marker based on the genera present in the region.

Molecularly distinct species in the Bangiales are assigned unique identifiers (codes) which consists of letters, numbers or a combination of these e.g. *Porphyra* GBR108. South African molecular entities have similarly been assigned codes based on their identification using the *rbc*L and nSSU genes. However, in the present study (Chapter 2), species were delimited based on the *cox*1 and *rbc*L genes for reasons mentioned above. The gene common to both studies (*rbc*L) did not always differentiate between closely related taxa and some discordance between markers and methods was found, particularly in *Porphyra*. For this reason, species were assigned new identifiers and later an attempt was made to match molecular entities from Jones *et al*. (2004) with the molecular species from

Chapter 2. Based on these comparisons species boundaries for a large majority of entities from Jones *et al*. (2004) were confirmed and additional species were recognized.

In Chapter 2, two widespread and abundant molecular species (RSAa & RSAb) were further divided into three and two lineages, respectively (RSAaa, RSAab, RSAac & RSAba, RSAbb). Only two of these lineages (RSAaa & RSAbb) were supported as being distinct species based on a 50% majority rule and supported by a multigene phylogeny. RSAab, RSac and RSba were only supported as distinct when one of the aforementioned methods was used (i.e. either the consensus or the multigene phylogeny). The molecular species RSAa and RSAb were represented by the largest number of specimens in the collection and were distributed over a wide geographic range. The further division of lineages within these molecular species could indicate some level of population level structure related to biogeography. This is particularly true for RSAb which formed two distinct haplotype groups on either side of Cape Point, a major biogeographic break along the South African coast. For this reason, lineages RSAab, RSAac and RSAba were not considered distinct species. Alternatively the *rbc*L gene could reflect a case of incomplete lineage sorting (polyphyletic lineages in the process of speciation), and species are therefore better captured by the faster evolving *cox*1 gene (Supplementary figure S1, S2).

Chapter 2 significantly contributed toward understanding the diversity in the Bangiales in South Africa, demonstrated the utility of analytical methods for studies of this type and has added to the large body of research on this topic globally.

In Chapter 3, the globally distributed and speciose genus *Pyropia* was assessed for the first time in South Africa since it was resurrected as a distinct genus in 2011. Since then, the application of molecular-assisted alpha taxonomy in the genus has resulted in accelerated species discovery worldwide. Seven species of *Pyropia* (all previously assigned to *Porphyra*) have been reported from the Benguela Marine Province in southern African (South Africa & Namibia). The diversity and identity of species of *Pyropia* from this region was assessed using an integrative taxonomic approach, including a multigene phylogeny, morphological and ecological data. This resulted in a species that was first collected on the South African coast 60 years ago being described as *Py. meridionalis* sp. nov. This species is associated with kelp, either growing on the kelp limpet, *Cymbula compressa* or epiphytically on kelp in the region: predominantly growing on *Ecklonia maxima* but may also grow on *E. radiata* or *Laminaria pallida*. The discovery of a new species from the subtidal environment, a relatively unexplored region in South Africa, highlights the possibility of other new subtidal species awaiting discovery. Similar to *Py. saldanhae* and *Py. aeodis*, *Py. meridionalis* is endemic to the Benguela Marine Province in southern Africa. Extended descriptions and further information on the distribution and ecology for *Py. saldanhae* and *Py. aeodis* were provided and photomicrographs were

presented for the first time for *Py. saldanhae.* Lastly, the occurrence of two widely distributed species, *Py. gardneri* and *Py. suborbiculata* could not be confirmed in this study (and was not confirmed in a previous study of the bladed Bangiales using molecular data by Jones *et al*. in 2004). The South African entity previously ascribed to *Py. gardneri* was shown to be the new species, here described as *Py. meridionalis*. The identity of *Py. suborbiculata* based on morpho-anatomical characters alone is tentative and requires further study. Phylogenetic affinities of southern African *Pyropia* in a global context using three unlinked loci (*cox*1, *rbc*L, nSSU) showed that all three species of *Pyropia* from southern African are endemic to the region but are not closely related to one another. This suggests that each species may have dispersed and speciated along this coastline independently. Although southern African species were recovered in separated clades, each species mostly shared close affinities with other species from the Southern Hemisphere in their respective clades. This supports the notion of historic connectivity in the Southern Ocean, but suggests that several dispersal events could have taken place during various times, as some clades were deeply divergent while others were more closely related.

In Chapter 4, additional molecular sequence data, morpho-anatomical and ecological characters were employed to further test for congruence of species boundaries for the 10 species of *Porphyra* identified in Chapter 2. Results from Chapter 3 indicate that South African *Porphyra* can be classified into two endemic cryptic species groups in South Africa. The first is a species-rich and genetically diverse *P. capensis* complex comprising eight morphologically cryptic and largely sympatric species along the west coast of South Africa (Benguela Marine Province). The second is a pair of cryptic sibling species along the south coast of South Africa (Agulhas Marine Province) which is morphologically and molecularly distinct from the *P. capensis* complex on the west coast. The second group was characterized by low genetic diversity with 82% of all individuals sharing a common haplotype. The more widely distributed and abundant of the cryptic pair was described as *P. agulhensis* sp. nov. Major patterns of genetic differentiation in the genus *Porphyra* (*P. capensis* complex/*P. agulhensis* duo) mirrored the Benguela/Agulhas transition zone, a region of known biogeographic and genetic change along the South African coastline. Furthermore, intraspecific phylogeographic structure in selected cryptic species was congruent with regions of higher intensity upwelling cells in the southern Benguela system, which has been hypothesized to act as semi-permeable genetic barriers. Similar processes further back in time could have been responsible for the high species diversity of *Porphyra* along the South African west coast.

Chapter 4 explored potential drivers influencing the evolution and genetic variation of *Porphyra* in South Africa. Two major drivers were tested; oceanographic processes and seawater temperature. Different processes that may have operated at different time scales were used to explain patterns of genetic variation in *Porphyra* in South Africa. Long term temperature differences were used to

explain major differences between cryptic species groups and could explain their distinct morphologies (south coast species are morphologically distinct from west coast species). Contemporary upwelling along the west coast of South Africa was used to explain within species differences (phylogeographic differences) in species within the PCC. This multidisciplinary approach allowed for plausible hypotheses to be tested and proposed.

In both Chapters 3 and 4 new species were described and the supplemental use of herbarium specimens aided in determining the distribution, seasonal occurrence and habitat association of species, as well as to determine whether species were recent invaders. This demonstrates the value of such records in taxonomic studies (Brodie *et al*., 2008b; Nelson et al., 2013, Chapter 3, 4). Herbarium records were also useful for confirming a range extension for *Py. saldanhae* and confirmed the unusual occurrence of species of the PCC along the south coast of South Africa during the winter of 2015. Sporadic upwelling events or cold spells along the south coast of South Africa during this time may have created optimal conditions for gametophytes of species in the PCC to grow. If this is true, then the dispersal of conchocelis may extend to the south coast of South Africa and growth of gametophytes may only occur in response to optimal environmental conditions. This requires further study.

For Chapters 3 and 4 appraisals of the genera *Pyropia* and *Porphyra* in South Africa using an integrative approach yielded some similar and some contrasting findings. In both chapters molecular data was used to resolve the mistaken identities of two species; *P. agulhensis* was recognized as distinct from *P. capensis* and *Py. gardneri* shown to be a misidentification of *Py. meridionalis*. However, findings in both chapters differ as many more species of *Porphyra* were found compared to *Pyropia*. In particular, extensive species diversity was concealed under the umbrella species *P. capensis*, while *Py. meridionalis* represents a case of taxonomic inflation (previously considered to represent four species). In general, these findings are contrary to Bangialean studies from around world, where many more species of *Pyropia* have been found compared to *Porphyra*. However, this is not surprising as the genus *Porphyra* appears to be speciose and more common in the Southern Hemisphere based on recent molecular studies (Sutherland *et al*., 2011; Guillemin *et al*., 2016).

The South African coast is now home to the second highest number of species of *Porphyra* in the world and is the only region in the Southern Hemisphere with two described species. Furthermore, *P. agulhensis* sp. nov. is the only species from this region (the Southern Hemisphere) to be described using both molecular and morphological data. The coasts of South Africa and two other Southern Hemisphere countries (Chile & New Zealand) are home to more two thirds of the total number of species of *Porphyra* globally. However, much of the Southern Ocean and most Southern Ocean Islands remain to be studied, which may help to clarify taxonomic relationships in this genus.

Global comparisons for *Pyropia* (Chapter 3) and *Porphyra* (Chapter 4) both support the hypothesis of past connectivity of red algae in the Southern Ocean; however based on genetic divergence this could have occurred several times throughout history. A much needed time-calibrated phylogeny will likely resolve this hypothesis and provide further insight.

In southern Africa, species of *Pyropia* are not only highly genetically divergent but are also morphologically divergent. This is in contrast to the recently diverging species of *Porphyra* in South Africa, in which molecular divergence has likely exceeded morphological divergence. This, rather than convergent evolution, is a more likely scenario because of the low genetic divergence between species of *Porphyra*. The recent and rapid species radiation in *Porphyra* in South Africa may explain its extensive distribution and high abundance. For *Pyropia*, a long evolutionary history may account for more pronounced genetic and morphological differences between species. Species of *Pyropia* in southern Africa occupy the lower eulittoral and sublittoral, both of which are often densely inhabited by a range of marine organisms. Furthermore, species of *Pyropia* are associated with particular habitat preferences. Competition and limited habitat may therefore explain the low abundance of *Pyropia* species compared to *Porphyra*. Competition is further supported by clearing experiments where the removal of *Scutellastra cochlear* (Born, 1778), a dominant low eulittoral limpet grazer, resulted in the growth of high densities of *P. saldanhae* (now *Py. saldanhae*; Joska in Stegenga *et al*., 1997). Other factors such as ecophysiological adaptation may also explain why *Porphyra* is more common and abundant than *Pyropia* in southern Africa.

Although this thesis fills a major research gap and may contribute to resolving phylogenetic relationships in future studies of the Bangiales, it also raises further questions about this enigmatic order.

Future research

Future studies on the Bangiales in southern Africa should extend sampling along the Benguela Marine Province, into Namibia, and beyond, into Angola. A preliminary assessment of species of *Porphyra* from Namibia based on the nSSU and ITS genes suggest that taxa in Namibia are distinct from those in South Africa (Kavishe, 2015; unpublished data). An attempt to compare nSSU data from the present study and those from Namibia confirms that some taxa are distinct while others are closely related and share an evolutionary history with South African species. All African specimens of *Porphyra* were recovered in a monophyletic clade based on the nSSU gene, although support was lacking. An analysis of faster evolving genes might offer clearer insights into the divergence of species of *Porphyra* along the Benguela Marine Province (South African & Namibia). Further assessment of morpho-anatomical traits of Namibian specimens may result in the description of more species from southern Africa or the addition of cryptic species to the PCC. This will also help confirm

distributional limits for species in the PCC and may even clarify taxonomic relationships between Chilean and South African species of *Porphyra*. Although, *Py. saldanahe*, *Py. aeodis* and *Py. meridionalis* are considered southern African species, sequence data of these species outside South Africa is lacking. Generation of such sequences will not only confirm the distribution of these species but, may also contribute to the understanding of the origin and evolution of African species of *Pyropia*. Extended sampling should also include various regions in the Southern Ocean where *P. capensis* has been recorded based on morphology. In particular exploring sub-antarctic islands in the South Atlantic where species are likely to have close affinities with southern Africa taxa (Chamberlain, 1965) are predicted to yield further phylogenetic clarification in *Porphyra*.

Future research along the South African coast will likely entail documenting temporal and spatial variability of cryptic species of *Porphyra* in relation to biogeography and upwelling. Extended sampling of specimens of *Pyropia* from different upwelling regions can also be used to determine if such events have similarly impacted the evolutionary history of this genus in South Africa as has been hypothesized for *Porphyra*. Although a large number of specimens was collected throughout the distribution range of the PCC and over different seasons, no site was revisited in different seasons (e.g. during the summer and winter for 1–2 years). Schweikert *et al*. (2012) found that some species of the bladed Bangiales in New Zealand occur seasonally while others were present throughout the year at particular localities. Such information remains unknown for the cryptic species of the PCC. Seasonal sampling at selected sites over a few years may also reveal rare species that may have not been encountered in the present study. Additional research could investigate whether different cryptic species differ eco-physiologically and if this can be linked to temporal and spatial patterns.

Very little is known about the new species *P. agulhensis* and *Py. meridionalis*. Both species appear to be abundant during the summer but this pattern as well as their abundance and detailed geographic distributional ranges remain to be further investigated. Ecophysiologcial studies might offer insight into the thermal tolerance of these species. This will be particularly interesting for *P. agulhensis* which is surrounded by eastern warming (east coast of South Africa) and western cooling (west coast of South Africa). Predictions about the distribution of this newly discovered and endemic species under various climate change scenarios can further be explored. Lastly, both species could be investigated for their potential cultivation in the nori industry. Both species consist of delicate blades, a property favoured for selection of species in the nori industry with prepared *P. agulhensis* tasting similar to nori (Rob Anderson personal observation 2017).

Dated phylogenies have become a useful tool to infer timelines for the evolution of species. Dates generated from these phylogenies can be used to propose evolutionary hypotheses that may coincide with the timing of major geological or oceanographic events. For example, if dates of divergence in the bladed Bangiales in South Africa are consistent with upwelling in the region then this could

strengthen the proposed evolutionary hypothesis (upwelling-driven speciation). However, no reliable dates exist for the Bangiales and until such time these become available, speciation events in South African taxa can only be inferred from net genetic divergence estimates.

Despite the already high species diversity of the Bangiales in South Africa, it is likely that further sampling may yield more species. This has similarly been suggested for the bladed Bangiales in New Zealand and Chile where high levels of diversity have already been found.

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SUPPLEMENTARY TABLES AND FIGURES

Table S1: Sample collection dates and sites.

Date	Site	Sample ID
18-05-2015	Port Nolloth	PN1-11
19-05-2015	Kleinzee	KZ1-7
20-05-2015	Hondeklipbaai	HK1-8
21-05-2015	Doringbaai	DB1-6
22-05-2015	Lamberts Bay	LB1-8
22-05-2015	Elands Bay	EB1-7
17-08-2014	Yzerfontein	YZ1-15
09-08-2014	Jacobsbaai	JC1-23
10-08-2014	Kraalbaai	KR1-8
10-08-2014	Tsaarsbaai	TS1-23
23-04-2015	Marcus Island	MI1-12
23-04-2015	Club Mykonos	CM1-3
23-01-2015	Ouderkraal	OD1
31-07-2015	Sea Point	SP1
16-08-2014	Kommetjie	KM1-8
07-01-2015	Kommetjie	KM9
24-02-2015	Soetwater	SW1
27-11-2014	Buffels bay	BB1-8
29-05-2014	Miller’s point	MP1-3
29-05-2014	Glencairn	GC1-9
29-05-2014	Muizenberg	MZ1-9
15-07-2015	Strandfontein	STD1-2
15-07-2015	Rooiels	RE1-11
18-07-2015	Pearly Beach	PB1-7
15-07-2015	Vermont, Hermanus	VM1-4
18-07-2015	De Kelders, Gansbaai	DKG1-4
16-07-2015	Suiderstrand	SS1-10
09-07-2015	Mossel Bay	MB1-4
09-07-2015	Herold's Bay	HB1-4
09-07-2015	Buffels bay, Knysna	BBK1-4
09-07-2015	Knysna Heads	KH1-5
06-12-2014	Nature's Valley	NV1
08-07-2015	Plettenberg Bay	PBB1-12
08-07-2015	Cape St. Franscis	CSF1-8
08-07-2015	Port Elizabeth	PE1-13
07-07-2015	Port Alfred	PA1-7
07-07-2015	East London	EL1-7

Table S2: Information on primers used and designed in this study.

Gene	Target species	Primers		Source
		Forward	***Reverse***	
***rbc*L**	*Porphyra* and *Pyropia*	KitoF	JrSR	Broom *et al*. 2010
***cox*1**	*Porphyra*	GSWR-MAGF: 5' TCIACYAAYCAYAAAGATATYGG3'	GSWR-MAGR: 5' ACTTCYGGRTGKCCAAAAAATC 3'	Saunders and Moore 2013
		Designed primers		**Based on**
***cox*1**	*Porphyra* (containing introns)	GSWR-MAGF: 5' TCIACYAAYCAYAAAGATATYGG3'	COXPor400: 5' GAA GAA GCT CCC GAT AAA 3'	Existing *cox*1 dataset for non-intron containing *Porphyra*
***cox*1**	*Pyropia saldanhae*	GSWR-MAGF: 5' TCIACYAAYCAYAAAGATATYGG3'	COXSal400R: 5' AGA AGA AGC TGA TAA ATG 3'	*cox*1 sequences of *Py. pulchra* (Hollenberg) S.C. Lindstrom and Hughey
***cox*1**	*Pyropia aeodis*	GSWR-MAGF: 5' TCIACYAAYCAYAAAGATATYGG3'	COXAeo500R: 5' GTC AAA ACA GGT ACT GCT AAT AAT A or 3'; COXPorR 400:5' GAAGAAGCTCCCGATAAATGC 3'	*cox*1 sequences of *Py. orbicularis* and *Py*. sp. FIA

Table S3: Accession numbers for all specimens acquired from GenBank for the *cox*1 and *rbc*L gene for trees in Figure S1-4.

Species	***rbc*L accession numbers**	***cox*1 accession numbers**
Pyropia abbottiae	AF168674.1; EU223024; JN028965.1	HM915228.1; HQ544959.1; HQ969860.1
Pyropia acanthophora	HQ605695; KJ852654.1	JN222750.1; KJ852653.1; KP998751.1
Pyropia aeodis	GU165843	
Pyropia bajacaliforniensis	KP904066.1; KP904065.1	
Pyropia brumalis	AF452426.1; EU223038	
Pyropia cinnamomea	EU521637	
Pyropia sp. collinsii		HM917381.1; JN028793.1; KJ961057.1
Pyropia columbiensis	KP904004.1; KP904003.1;KP904002.1	
Pyropia columbina	GU046412.1; GU046423; GU046431.1	
Pyropia conwayae	EU223045; AF452427.1	HQ699185.1; HQ699187.1; HQ699188.1
Pyropia cf. crassa	HQ687518	
Pyropia dentata	HQ687520; AB118579.1; AB287927.1; GQ427223.1	
Pyropia denticulata	HQ687521	

Pyropia elongata	JN847254.1; KC347609.1; KJ182952.1; FJ817088.1	
Pyropia fallax	GU319865; AF168661.1; JN028967.1; EU223056.1	JN028601.1; HQ544741.1; HQ969862.1
Pyropia francisii	HQ687537.1	
Pyropia fucicola	EU223088; AF452430.1; JN028970.1	JN028613.1; JN028618.1; JN028626.1
Pyropia gardneri	EU223096; HQ687522; JN028971.1	JN028643.1; HM915292.1; HQ545220.1
Pyropia haitanensis	AB118585; KP266616.1; GQ427202.1	KP266600.1
Pyropia hiberna	GU319866	
Pyropia hollenbergii	HQ687523; JQ900559.1; AY794401.1	
Pyropia ishigecola	HQ687524.1; GQ427224.1; GQ427225.1	
Pyropia kanakaensis	EU223099; AF168662.1; JN028978.1	
Pyropia kanyakumariensis		KP998745.1; KP998747.1; KP998748.1
Pyropia katadae	AB118583.1; DQ630034.1; HQ687525	
Pyropia kinositae	EU521641; AB366139.1; AB366145.1	
Pyropia koreana	HQ728198; GU165841; KC347610.1; KJ182947.1 KM078727.1	
Pyropia kuniedae	HQ728200	
Pyropia kurogii	AF452432.1; JN028980.1; EU223105; HQ687526	JN028654.1; JN028655.1; HQ699177.1
Pyropia lacerata	HQ687527	
Pyropia lanceolata	GU319867; GU319868; AF452433.1	KR139854.1
Pyropia leucosticta	AF271078.1; DQ308424.1; JN028982.1; HQ687528	AM943398.1; JN028656.1; DQ442910.1; HM916363.1; KJ961056.1
Pyropia montereyensis	KP903977.1; KP903975.1; KP903972.1	
Pyropia moriensis	EU521645	
Pyropia nereocystis	AF168672.1; JN028983.1; JN028984.1	JN028685.1; JN028686.1; HQ699186.1
Pyropia nitida	KP876025.1	
Pyropia njordii	JN847258.1; JN847259.1; KF478757.1	JN028690.1; JN028691.1; JN847326.1
Porphyra oligospermatangia	GQ427213.1	
Pyropia onoi	HQ687529	
Pyropia orbicularis	KF479481.1; KF479482.1; KF479501.1	KP781667.1; KP781685.1; KP781697.1
Pyropia parva	KJ182946.1	
Pyropia peggicovensis	JN028992.1; JN028991.1; JN028989.1	
Pyropia pendula	HQ687530; JQ900553.1; JQ900557.1	
Pyropia sp. **GCI**	JQ900562.1; JQ900561.1; JQ900560.1	
Pyropia sp. **GCII**	JQ900565.1	

***Pyropia sp.* GCIII**	JQ900556.1; JQ900556.1; JQ900554.1	
Pyropia perforata	EU223127; AF452438.1; GU046416.1; JN028996.1	JN028708.1; HQ919270.1; KM254424.1
Pyropia plicata	GU046410.1	
Pyropia protolanceolata	KP904006.1; KP904005.1	
Pyropia pseudolanceolata	KP904060.1; EU223157.1	HQ699176.1
Pyropia pseudolinearis	HQ728196; AB118581.1; AF452441.1; HQ687531	
Pyropia pulchella	HQ687532; GU046419.1	
Pyropia raulaguilarii	JQ684700.1; JQ684701.1; JQ684702.1	
Pyropia rakiura	EU521646	
Pyropia saldanhae	GU165838	
Pyropia seriata	HQ687533	
Pyropia smithii	EU223224; JN028997.1; JN028998.1	JN028779.1; HQ969857.1; HQ969859.1
Pyropia spiralis	HQ605696	JN222758.1; JN222759.1; JN222760.1
Pyropia stamfordensii		JN028798.1
Pyropia suborbiculata	HQ728201; AF078743.1; AY028523.1; DQ630041.1 EU521647.1; GQ427221.1; AB118580.1; JQ327838.1	
Pyropia tanegashimensis	HQ687542; AB671541.1	JN222752.1
Pyropia tenera	HQ687543; AB118576.1; AB243206.1	AB477377.1
Pyropia tenuipedalis	EU521649 AB287951.1	
Pyropia thulaea	JN847268.1; KF478758.1	
Pyropia thuretii	HQ687519; JN029006.1; JN029007.1	JN028799.1; JN028800.1; JN028801.1
Pyropia torta	EU223236; AF452445.1; JN029008.1	JN028802.1
Pyropia vietnamensis	HQ687544	JN028802.1; HQ422669.1; KM977745.1
Pyropia viridident ata	EU521650	
Pyropia yezoensis	HQ687545; AB118574.1; DQ227860.1; FJ610255.1 GQ427214.1	AB477378.1; JN028803.1
		KC782859.1
***'Porphyra' sp.* MDJL**		HQ699204.1
***Pyropia sp.* FIA**		KP781603.1
***Pyropia sp.* CHJ**		KP781648.1
***Pyropia sp.* CHI**		KP781620.1
***Pyropia sp.* CHH**		KP781676.1
'*Porphyra' sp.* ARS		HQ423032.1
***Pyropia sp.* ARC-P-207**		KP998749.1
***Pyropia sp.* 6POR**	JN029003.1	JN028792.1

***Pyropia sp.* 1POR**		JN028788.1
***Pyropia sp.* 2Cal**		JN028791.1
***Pyropia sp.* 1Cal**		JN028782.1
***Pyropia sp.* CHK**		KP781619.1
***Pyropia sp.* Antar68**	HQ605698	
***Pyropia sp.* DRB**	HQ605698	
***Pyropia sp.* FAL**	HQ687535	
***Pyropia sp.* MIG**	HQ687536	
***Pyropia sp.* Piaui**	HQ605697	
***Pyropia sp.* ROS125**	HQ687538	
***Pyropia sp.* SMR**	HQ687539	
***Pyropia sp.* STI**	HQ687540	
***Pyropia sp.* WRO**	HQ687541	
***Pyropia sp.* ZLI**	GU165839.1	
Pyropia sp.	AB818920.1; JN847267.1	
Porphyra corallicola	JN028943.1	JN028496.1
Porphyra dioica	HQ687546; AF081291.2; JN703282.1; LN877843.1	DQ442889.1; HQ699230.1 JN847312.1
Porphyra linearis	AF168673.1; HQ687547; JN787105.1; KJ182953.1	JN847314.1; JN847316.1; JN847315.1
Porphyra lucasii	AY139687	
Porphyra oligospermatangia	GQ427213.1; GQ427227.1; GQ427226.1	
Porphyra plocamiestris	AF168671.1	
Porphyra mumfordii	AF452434.1; EU223111.1; JN028947.1	JN028497.1; JN028498.1; HQ699181.1
Porphyra purpurea	HQ687516; AF168668.1; DQ406598.1; JN831094.1; KF478754.1	HM918821.1; JN028514.1; JN847317.1
Porphyra umbilicalis	HQ687559; AB118584.1; AF078747.1; AY028538.1 JN028956.1; KF478756.1	HM918792.1; HQ919628.1; JN028558.1; DQ442892.1; AM943404.1; JN847309.1
***'Porphyra' sp.* AMM06SF2347**		AM943400.1
***Porphyra sp.* 1FIH**		JN028551.1
***Porphyra sp.* FIH**		KP781688.1; KP781688.1
***Porphyra sp.* CHB**		KP781663.1
***Porphyra sp.* CHC**		KP781644.1; KP781645.1; KP781684.1
***Porphyra sp.* CHD**		KP781673.1
***Porphyra sp.* CHF**		KP781693.1; KP781694.1; KP781695.1

Porphyra sp.	**FIB**	GU165840
Porphyra sp.	**FIG**	GU165885
Porphyra sp.	**GDM143**	GU046415
Porphyra sp.	**GRB108**	GU214021
Porphyra sp.	**GRB145**	HQ687548
Porphyra sp.	**GRB178**	HQ687549
Porphyra sp.	**GRB287**	HQ687550
Porphyra sp.	**GRB368**	HQ687551
Porphyra sp.	**GRB488**	GU046405
Porphyra sp.	**JBCH26A**	HQ687552
Porphyra sp.	**LGD030**	GU046409
Porphyra sp.	**MTR**	HQ687553
Porphyra sp.	**OSK**	HQ687554
Porphyra sp.	**SBA190**	GU046414
Porphyra sp.	**SIR242**	GU046417
Porphyra sp.	**TAS333**	GU046427
Porphyra sp.	**TCH243**	GU046418
Porphyra sp.	**WLR260**	GU165837
Porphyra sp.	**ZBS**	HQ687555
Porphyra sp.	**ZCE965**	GU046424
Porphyra sp.	**ZDR966**	GU046425
Porphyra sp.	**ZGR**	HQ687556
Porphyra sp.	**ZIR901**	GU214022
Porphyra sp.	**ZPP**	HQ687557
Porphyra sp.	**ZSM**	HQ687558
Porphyra sp.		EU223172; AF168664.1
		AB118586.1
		AB287953.1
		AB366146.1
		AB455541.1
		AF168666.1
		DQ787556.1
		EU223013.1
		GU046403.1
		HQ687548.1

	JN028950.1 JN787112.1

Table S4: Haplotype list of sequences used for phylogenetic analyses for both genera, and for three genes.

***Porphyra cox*1**		
Haplotype	**No.**	**Contains**
***Porphyra linearis* JN847314**	2	*Porphyra linearis* JN847316
***Porphyra mumfordii* JN028497**	2	*Porphyra mumfordii* JN028498
***Porphyra purpurea* HM918821**	2	*Porphyra purpurea* JN028514
***Porphyra umbilicalis* HM918792**	3	*Porphyra umbilicalis* HQ919628
		Porphyra umbilicalis JN028558
***Porphyra* sp. FIH KP781696.1**	3	*Porphyra* sp. FIH KP781686.1
		Porphyra sp. FIH KP781681.1
***Porphyra* sp. CHF KP781695.1**	2	*Porphyra* sp. CHF KP781694.1
***Porphyra* sp. CHC KP781645.1**	2	*Porphyra* sp. CHC KP781644.1
***Porphyra* sp. BBK1 KX852772**	39	*Porphyra* sp. BBK2 KX852773
		Porphyra sp. BBK3 KX852774
		Porphyra sp. BBK4 KX852775
		Porphyra sp. CSF4 KX852787
		Porphyra sp. CSF5 KX852788
		Porphyra sp. CSF6 KX852789
		Porphyra sp. CSF8 KX852790
		Porphyra sp. HB1 KX852819
		Porphyra sp. HB2 KX852820
		Porphyra sp. HB3 KX852821
		Porphyra sp. HB4 KX852822
		Porphyra sp. KH2 KX852831
		Porphyra sp. KH3 KX852832
		Porphyra sp. KH5 KX852834
		Porphyra sp. MB1 KX852859
		Porphyra sp. MB2 KX852860
		Porphyra sp. MB4 KX852861
		Porphyra sp. PA2 KX852875
		Porphyra sp. PA3 KX852876
		Porphyra sp. PA4 KX852877

		Porphyra sp. PA5 KX852878
		Porphyra sp. PBB1 KX852880
		Porphyra sp. PBB10 KX852889
		Porphyra sp. PBB11 KX852890
		Porphyra sp. PBB12 KX852891
		Porphyra sp. PBB2 KX852881
		Porphyra sp. PBB5 KX852884
		Porphyra sp. PBB7 KX852886
		Porphyra sp. PBB8 KX852887
		Porphyra sp. PBB9 KX852888
		Porphyra sp. PE1 KX852899
		Porphyra sp. PE13 KX852909
		Porphyra sp. PE3 KX852901
		Porphyra sp. PE4 KX852902
		Porphyra sp. PE5 KX852903
		Porphyra sp. PE6 KX852904
		Porphyra sp. PE7 KX852905
		Porphyra sp. PE8 KX852906
***Porphyra* sp. BB1 KX852776**	12	*Porphyra* sp. BB5 KX852779
		Porphyra sp. BB6 KX852780
		Porphyra sp. KM1 KX852835
		Porphyra sp. RE6 KX852922
		Porphyra sp. TS12 KX852943
		Porphyra sp. VM2 KX852946
		Porphyra sp. VM3 KX852947
		Porphyra sp. VM4 KX852948
		Porphyra sp. GC6 KX852817
		Porphyra sp. MP3 KX852872
		Porphyra sp. RE1 KX852920
***Porphyra* sp. BB3 KX852778**		
***Porphyra* sp. BB4 KX852777**		
***Porphyra* sp. BB7 KX852781**		
***Porphyra* sp. CM1 KX852782**	3	*Porphyra* sp. KR4 KX852844
		Porphyra sp. KR6 KX852846
***Porphyra* sp. CM3 KX852783**	4	*Porphyra* sp. KR2 KX852842

		Porphyra sp. KR3 KX852843
		Porphyra sp. KR5 KX852845
***Porphyra* sp. CSF1 KX852784**		
***Porphyra* sp. CSF2 KX852785**	5	*Porphyra* sp. KM2 KX852836
		Porphyra sp. KM3 KX852837
		Porphyra sp. KM4 KX852838
		Porphyra sp. OD1 KX852873
***Porphyra* sp. CSF3 KX852786**		
***Porphyra* sp. DB1 KX852791**	13	*Porphyra* sp. DB2 KX852792
		Porphyra sp. HK5 KX852826
		Porphyra sp. HK6 KX852827
		Porphyra sp. HK8 KX852829
		Porphyra sp. KS1 KX852848
		Porphyra sp. KS6 KX852852
		Porphyra sp. PN11 KX852919
		Porphyra sp. PN2 KX852910
		Porphyra sp. PN3 KX852911
		Porphyra sp. PN5 KX852913
		Porphyra sp. PN6 KX852914
		Porphyra sp. PN7 KX852915
***Porphyra* sp. DB3 KX852793**	9	*Porphyra* sp. DB5 KX852795
		Porphyra sp. DB6 KX852796
		Porphyra sp. EB3 KX852803
		Porphyra sp. EB4 KX852804
		Porphyra sp. EB5 KX852805
		Porphyra sp. EB7 KX852806
		Porphyra sp. KH1 KX852830
		Porphyra sp. LB4 KX852856
***Porphyra* sp. DB4 KX852794**	4	*Porphyra* sp. TS1 KX852937
		Porphyra sp. TS3 KX852938
		Porphyra sp. TS5 KX852939
***Porphyra* sp. DKG1 KX852797**		
***Porphyra* sp. DKG3 KX852798**	15	*Porphyra* sp. DKG4 KX852799
		Porphyra sp. DKG7 KX852802

		Porphyra sp. PB4 KX852895
		Porphyra sp. PB5 KX852896
		Porphyra sp. PB6 KX852897
		Porphyra sp. PB7 KX852898
		Porphyra sp. SS1 KX852925
		Porphyra sp. SS10 KX852934
		Porphyra sp. SS3 KX852927
		Porphyra sp. SS4 KX852928
		Porphyra sp. SS6 KX852930
		Porphyra sp. SS7 KX852931
		Porphyra sp. SS8 KX852932
		Porphyra sp. SS9 KX852933
***Porphyra* sp. DKG5 KX852800**	7	*Porphyra* sp. EL1 KX852807
		Porphyra sp. EL2 KX852808
		Porphyra sp. EL3 KX852809
		Porphyra sp. EL5 KX852811
		Porphyra sp. EL6 KX852812
		Porphyra sp. EL7 KX852813
***Porphyra* sp. DKG6 KX852801**	2	*Porphyra* sp. GC4 KX852816
***Porphyra* sp. EL4 KX852810**		
***Porphyra* sp. GC1 KX852814**	2	*Porphyra* sp. GC2 KX852815
***Porphyra* sp. GC7 KX852818**	2	*Porphyra* sp. PBB6 KX852885
***Porphyra* sp. HK1 KX852823**	2	*Porphyra* sp. HK4 KX852825
***Porphyra* sp. HK2 KX852824**	7	*Porphyra* sp. HK7 KX852828
		Porphyra sp. KS3 KX852850
		Porphyra sp. LB8 KX852858
		Porphyra sp. PN4 KX852912
		Porphyra sp. PN8 KX852916
		Porphyra sp. PN9 KX852917
***Porphyra* sp. KH4 KX852833**		
***Porphyra* sp. KM5 KX852839**	2	*Porphyra* sp. KM7 KX852841
***Porphyra* sp. KM6 KX852840**		
***Porphyra* sp. KR7 KX852847**		
***Porphyra* sp. KS2 KX852849**	4	*Porphyra* sp. LB2 KX852855
		Porphyra sp. LB7 KX852857

		Porphyra sp. PN10 KX852918
***Porphyra* sp. KS5 KX852851**		
***Porphyra* sp. KS7 KX852853**		
***Porphyra* sp. LB1 KX852854**		
***Porphyra* sp. LB5 KX852957**		
***Porphyra* sp. LB6 KX852958**		
***Porphyra* sp. MB3 KX852861**		
***Porphyra* sp. MI1 KX852863**	13	*Porphyra* sp. MI10 KX852870
		Porphyra sp. MI11 KX852871
		Porphyra sp. MI2 KX852864
		Porphyra sp. MI4 KX85265
		Porphyra sp. MI5 KX852866
		Porphyra sp. MI6 KX852867
		Porphyra sp. TS13 KX852944
		Porphyra sp. TS8 KX852940
		Porphyra sp. TS9 KX852941
		Porphyra sp. TS10 KX852942
		Porphyra sp. YZ12 KX852953
		Porphyra sp. YZ13 KX852954
***Porphyra* sp. MI8 KX852868**		
***Porphyra* sp. MI9 KX852869**		
***Porphyra* sp. PA1 KX852874**		
***Porphyra* sp. PA6 KX852879**		
***Porphyra* sp. PB1 KX852892**		
***Porphyra* sp. PB2 KX852893**	2	*Porphyra* sp. PB3 KX852894
***Porphyra* sp. PBB3 KX852882**	2	*Porphyra* sp. PBB4 KX852883
***Porphyra* sp. PE2 KX852900**		
***Porphyra* sp. PE9 KX852907**		
***Porphyra* sp. PE12 KX852908**		
***Porphyra* sp. RE5 KX852921**		
***Porphyra* sp. RE7 KX852923**		
***Porphyra* sp. SP1 KX852924**	2	*Porphyra* sp. YZ11 KX852952
***Porphyra* sp. SS2 KX852926**		
***Porphyra* sp. SS5 KX852929**		
***Porphyra* sp. STD1 KX852935**		

***Porphyra* sp. STD2 KX852936**		
***Porphyra* sp. VM1 KX852945**		
***Porphyra* sp. YZ15 KX852955**	4	*Porphyra* sp. YZ3 KX852949
		Porphyra sp. YZ4 KX852950
		Porphyra sp. YZ7 KX852951
	***Porphyra* rbcL**	
Haplotype	**No.**	**Contains**
***Porphyra oligospermatangia* GQ427227**	2	*Porphrya oligospermatangia* GQ427226
***Porphyra* sp. AB118586**	2	*Porphyra* sp. AB287953
***Porphyra* sp. BBK 3 KX852959**	6	*Porphyra* sp. CSF1 KX852964
		Porphyra sp. HB1 KX852972
		Porphyra sp. KH2 KX852975
		Porphyra sp. PE13 KX852994
		Porphyra sp. PE5 KX852995
***Porphyra* sp. GC6 KX852960**		
***Porphyra* sp. GC7 KX852961**		
***Porphyra* sp. CM1 KX852962**	2	*Porphyra* sp. CM2 KX852963
***Porphyra* sp. DB4 KX852965**		
***Porphyra* sp. DKG1 KX852966**		
***Porphyra* sp. DKG5 KX852967**		
***Porphyra* sp. DKG6 KX852968**		
***Porphyra* sp. EB3 KX852969**		
***Porphyra* sp. EB6 KX852970**		
***Porphyra* sp. EL3 KX852971**		
***Porphyra* sp. GC5 KX853016**		
***Porphyra* sp. HK3 KX852973**	4	*Porphyra* sp. KH1 KX852974
		Porphyra sp. PN1 KX852996
		Porphyra sp. PN5 KX852997
***Porphyra* sp. KM1 KX852976**	6	*Porphyra* sp. KM5 KX852979
		Porphyra sp. KR2 KX852980
		Porphyra sp. KR5 KX852981
		Porphyra sp. RE1 KX852999
		Porphyra sp. VM1 KX853011
***Porphyra* sp. KM2 KX852977**	2	*Porphyra* sp. OD1 KX852989

***Porphyra* sp. KM4 KX852978**		
***Porphyra* sp. KR1 KX853017**	2	*Porphyra* sp. MP1 KX853018
***Porphyra* sp. KS2 KX852982**	2	*Porphyra* sp. LB5 KX852984
***Porphyra* sp. KS4 KX852983**		
***Porphyra* sp. MI5 KX852985**	7	*Porphyra* sp. MI8 KX852986 *Porphyra* sp. MI9 KX852987 *Porphyra* sp. TS9 KX853010 *Porphyra* sp. YZ3 KX853013 *Porphyra* sp. YZ4 KX853014 *Porphyra* sp. YZ15 KX853015
***Porphyra* sp. MP2 KX853019**		
***Porphyra* sp. MP3 KX852988**		
***Porphyra* sp. MZ8 KX853020**		
***Porphyra* sp. PA3 KX852990**	2	*Porphyra* sp. PBB7 KX852993
***Porphyra* sp. PBB3 KX852991**	2	*Porphyra* sp. PBB6 KX852992
***Porphyra* sp. PN8 KX852998**		
***Porphyra* sp. SP1 KX853000**		
***Porphyra* sp. SS1 KX853001**	8	*Porphyra* sp. SS3 KX853002 *Porphyra* sp. SS4 KX853003 *Porphyra* sp. SS5 KX853004 *Porphyra* sp. SS6 KX853005 *Porphyra* sp. SS7 KX853006 *Porphyra* sp. SS8 KX853007 *Porphyra* sp. SS9 KX853008
***Porphyra* sp. SS2 KX853021**		
***Porphyra* sp. STD1 KX853009**		
***Porphyra* sp. TS8 KX853022**		
***Porphyra* sp. VM3 KX853012**		
***Porphyra* sp. YZ1 KX853023**	2	*Porphyra* sp. YZ2 KX853024
***Porphyra* sp. YZ5 KX853025**		
***Porphyra* sp. YZ14 KX853026**		

***Porphyra* nSSU**		
Haplotype	**No.**	**Contains**
***Porphyra* sp. CM1**		

***Porphyra* sp. EB3**		
***Porphyra* sp. PBB3**		
***Porphyra* sp. KM5**		
***Porphyra* sp. KH1**		
***Porphyra* sp. BB4**		
***Porphyra* sp. DKG6**		
***Porphyra* sp. KS2**		
***Porphyra* sp. NV1**		
***Porphyra* sp. NV2**		
***Porphyra* sp. PE5**		
***Porphyra* sp. STD1**		
***Porphyra* sp. SHB5**		
***Porphyra* sp. KEM1**		
	***Pyropia cox*1**	
Haplotype	**No.**	**Contains**
***Pyropia fallax* JN028601**	3	*Pyropia fallax* HQ544741
		Pyropia fallax HQ969862
***Pyropia gardneri* JN028643**	2	*Pyropia gardneri* HQ545220
***Pyropia leucosticta* JN028656**	2	*Pyropia leucosticta* HM916363
***Pyropia nereocystis* JN028686**	2	*Pyropia nereocystis* HQ699186
***Pyropia smithii* HQ969857**	2	*Pyropia smithii* HQ969859
***Pyropia spiralis* JN222758**	3	*Pyropia spiralis* JN222759
		Pyropia spiralis JN222760
***Pyropia thuretii* JN028799**	3	*Pyropia thuretii* JN028800
		Pyropia thuretii JN028801
***Pyropia* sp. 1Cal JN028782**	2	*Pyropia* sp. 1Cal JN028784
***Pyropia* sp. 1Cal JN028784**	2	*Pyropia* sp. 1Cal JN028784
***Pyropia* sp. CHI KP781620**	3	*Pyropia* sp. CHI KP781621
		Pyropia sp. CHI KP781622
***Pyropia* sp. YZ10 KY814926**	2	*Pyropia* sp. MI12
***Pyropia* sp. JC4 KY814931**	2	*Pyropia* sp. TS20 KY814933
***Pyropia* sp. RE2 KY814939**	2	*Pyropia* sp. RE9 KY814941
***Pyropia* sp. KZ8**	3	*Pyropia* sp. KZ9-10
***Pyropia* sp. HK9**	3	*Pyropia* sp. HK10-11
***Pyropia* sp. DB7**	3	*Pyropia* sp. DB8-9

***Pyropia* sp. KZ8**	3	*Pyropia* sp. KZ9-10
***Pyropia* sp. PTN1**	3	*Pyropia* sp. PTN2-3
***Pyropia* sp. MZ11**		
***Pyropia* sp. MZ12**		
	***Pyropia rbc*L**	
Haplotype	**No.**	**Contains**
***Pyropia columbiensis* KP904003**	2	*Pyropia columbiensis* KP904002
***Pyropia conwayae* EU223045**	2	*Pyropia conwayae* EU223047
***Pyropia dentata* AB118579**	2	*Pyropia dentata* AB287927
***Pyropia montereyensis* KP903977**	3	*Pyropia montereyensis* KP903975
		Pyropia montereyensis KP903972
***Pyropia nereocystis* JN028983**	2	*Pyropia nereocystis* JN028984
***Pyropia oligospermatangia* GQ427227**		*Pyropia oligospermatangia* GQ427226
***Pyropia orbicularis* KF479482**	2	*Pyropia orbicularis* KF479501
***Pyropia peggicovensis* JN028991.1**	2	*Pyropia peggicovensis* JN028989.1
***Pyropia suborbiculata* AF078743**	2	*Pyropia suborbiculata* AY028523
***Pyropia* sp. AB118586**	2	*Pyropia* sp. AB287953
***Pyropia* sp. CHK KP781748.1**	3	*Pyropia* sp. CHK KP781747.1
		Pyropia sp. CHK KP781746.1
***Pyropia* sp. CHI KP781715.1**	2	*Pyropia* sp. CHI KP781713.1
***Pyropia* sp. GCI JQ900562**	3	*Pyropia* sp. GCI JQ900561
		Pyropia sp. GCI JQ900560
***Pyropia* sp. GCIII JQ900555**	2	*Pyropia* sp. GCIII JQ900554
***Pyropia* sp. RE2 KY814947**	2	*Pyropia* sp. RE10 KY814950
***Pyropia aeodis* SB4**		
***Pyropia aeodis* SB5**		
***Pyropia meridionalis* KZ8**		
***Pyropia meridionalis* HK9**		
***Pyropia meridionalis* DB7**		
***Pyropia meridionalis* KZ8**		
***Pyropia meridionalis* PTN1**		

***Pyropia meridionalis* SW2** ***Pyropia meridionalis* MZ11** ***Pyropia meridionalis* MZ12** ***Pyropia saldanhae* HK12** ***Pyropia saldanhae* MZ14** ***Pyropia saldanhae* JC36**	1	*Pyropia* sp. MZ11b
***Pyropia* nSSU**		
***Pyropia aeodis* SB5** ***Pyropia meridionalis* HK9** ***Pyropia meridionalis* DB7** ***Pyropia meridionalis* KZ8** ***Pyropia meridionalis* SW1** ***Pyropia meridionalis* SW3** ***Pyropia meridionalis* PTN1** ***Pyropia meridionalis* PTN2** ***Pyropia meridionalis* MZ11** ***Pyropia meridionalis* MZ12** ***Pyropia saldanhae* MZ14** ***Pyropia saldanhae* JC4** ***Pyropia saldanhae* JC36**		

Table S5: Accession numbers for all specimens acquired from GenBank for the *cox*1, *rbc*L and nSSU genes for the global phylogeny in Figure 2

Species	***cox*1**	***rbc*L**	**nSSU**	**Source**
Bangia atropurpurea NL	DQ442887	AF169330	AF169341	Sutherland et al. 2011
Bangia sp. IE		AF043371	AF043365	Sutherland et al. 2011
'*Bangia*' *fuscopurpurea* CA	JN028459	EU289018	EU289023	Sutherland et al. 2011
'*Bangia*' *fuscopurpurea* JP		HQ687502	HQ687561	Sutherland et al. 2011
'*Bangia*' *fuscopurpurea* FR		AF168659	AF175535	Sutherland et al. 2011

'*Bangia*' *fuscopurpurea* TW	AF168654	AF175529	Sutherland et al. 2011
'*Bangia*' *fuscopurpurea* WA	AF169329	AF169336	Sutherland et al. 2011
'*Bangia*' *gloiopeltidicola*	HQ687503	HQ687563	Sutherland et al. 2011
'*Bangia*'*maxima*	EU289020	EU289025	Sutherland et al. 2011
'*Bangia*' sp. BC Can	AF043376	AF043359	Sutherland et al. 2011
'*Bangia*' sp. BCH	HQ687504	AY184335	Sutherland et al. 2011
'*Bangia*'sp. BFK	HQ687505	AY184338	Sutherland et al. 2011
'*Bangia*' sp. BGA	HQ687506	AY184341	Sutherland et al. 2011
'*Bangia*' sp. BHH	GU046404	AY184339	Sutherland et al. 2011
'*Bangia*' sp. BJB	HQ687507	AY184337	Sutherland et al. 2011
'*Bangia*' sp. BMW	HQ687508	AY184344	Sutherland et al. 2011
'*Bangia*' sp. BNS	HQ687509	AY184345	Sutherland et al. 2011
'*Bangia*' sp. BPL	HQ687510	AH015107.2	Sutherland et al. 2011
'*Bangia*' sp. BRM	HQ687511	HQ687562	Sutherland et al. 2011
'*Bangia*' sp. BWP	EU570051	AY184348	Sutherland et al. 2011
'*Bangia*' sp. CH620	HQ728203	HQ728195	Sutherland et al. 2011
'*Bangia*' sp. Can	AF043372	AF043360	Sutherland et al. 2011
'*Bangia*' sp. NWT	AF043366	AF043355	Sutherland et

				al. 2011
'*Bangia*' sp. OR		AF043367	AF043358	Sutherland et al. 2011
'*Bangia*' SB		EU289019	EU289024	Sutherland et al. 2011
'*Bangia*' sp. TX		AF043377	AF043361	Sutherland et al. 2011
'*Bangia*' *vermicularis*	HQ699182	EU289022	EU289027	Sutherland et al. 2011
Boreophyllum aestivale	JN028481	EU223033	GU319836	Sutherland et al. 2011
Boreophyllum birdiae	HM916391	AY180909	HQ709388	Sutherland et al. 2011
Clymene coleana		FJ263672	AF136423	Sutherland et al. 2011
Clymene sp. OTA		GU214023	GU214024	Sutherland et al. 2011
Clymene sp. TTS		HQ687514	HQ687565	Sutherland et al. 2011
Dione arcuata		EU570052	AY465354	Sutherland et al. 2011
Fuscifolium papenfussii	JN028494	EU223120	GU319855	Sutherland et al. 2011
Fuscifolium tasa	HQ699253	EU223226	GU319862	Sutherland et al. 2011
Lysithea adamsiae		HQ687515	HQ687566	Sutherland et al. 2011
Minerva aenigmata		EU570053	AY465355	Sutherland et al. 2011
Miuraea migitae		EU521643	EU521642	Sutherland et al. 2011
Porphyra dioica	JN847312	HQ687546	HQ687579	Sutherland et al. 2011
Porphyra linearis	JN847314	HQ687547	HQ687580	Sutherland et al. 2011

Porphyra lucasii		AY139687	AY139685	Sutherland et al. 2011
Porphyra purpurea	HM918821	HQ687516	HQ687567	Sutherland et al. 2011
Porphyra sp. FIB		GU165840	AY909598	Sutherland et al. 2011
Porphyra sp. FIG		GU165885	GU165881	Sutherland et al. 2011
Porphyra sp. GDM		GU046415	AY909597	Sutherland et al. 2011
Porphyra sp. GRB108		GU214021	AF136420	Sutherland et al. 2011
Porphyra sp. GRB145		HQ687548	AY184349	Sutherland et al. 2011
Porphyra sp. GRB178		HQ687549	AY909603	Sutherland et al. 2011
Porphyra sp. GRB287		HQ687550	AY909595	Sutherland et al. 2011
Porphyra sp. GRB368		HQ687551	AY292639	Sutherland et al. 2011
Porphyra sp. GRB488		GU046405	AY184350	Sutherland et al. 2011
Porphyra sp. JBCH26A (CHF)	KP781693	HQ687552	HQ687581	Sutherland et al. 2011
Porphyra sp. LGD		GU046409	AF136422	Sutherland et al. 2011
Porphyra sp. MTR		HQ687553	HQ687582	Sutherland et al. 2011
Porphyra sp. OSK		HQ687554	AY909593	Sutherland et al. 2011
Porphyra sp. SBA		GU046414	AY909589	Sutherland et al. 2011
Porphyra sp. SIR		GU046417	AY909588	Sutherland et al. 2011
Porphyra sp. TAS		GU046427	AY909585	Sutherland et

				al. 2011
Porphyra sp. WLR		GU165837	AY292645	Sutherland et al. 2011
Porphyra sp. RSAaa (ZBS)	KX852803	HQ687555	AY292626	Sutherland et al. 2011
Porphyra sp. RSAbb (ZCE)	KX852882	GU046424	AY292627	Sutherland et al. 2011
Porphyra sp. RSAba (ZDR)	KX852929	GU046425	AY292628	Sutherland et al. 2011
Porphyra sp. RSAab (ZGR)	KX852782	HQ687556	AY292631	Sutherland et al. 2011
Porphyra sp. RSAe (ZIR)	KX852868	GU214022	AY292632	Sutherland et al. 2011
Porphyra sp. RSAi (ZPP)	KX852903	HQ687557	AY292636	Sutherland et al. 2011
Porphyra sp. ZSM		HQ687558	HQ687583	Sutherland et al. 2011
Porphyra umbilicalis	HM918792	HQ687559	HQ687584	Sutherland et al. 2011
Pseudobangia kaycoleia	HQ699190		AF043364	Sutherland et al. 2011
Pyropia abbottiae	HM915228	EU223024	GU319835	Sutherland et al. 2011
Pyropia acanthophora	KP998751	HQ605695	L26197	Sutherland et al. 2011
Pyropia aeodis	KY799110	GU165843	AY292624	Sutherland et al. 2011
Pyropia brumalis		EU223038	GU319837	Sutherland et al. 2011
Pyropia cf. *crassa*		HQ687518	HQ687569	Sutherland et al. 2011
Pyropia sp. *pseudolinearis*		EU223172	GU319858	Sutherland et al. 2011
Pyropia cf. *thuretii*	JN028799	HQ687519	HQ687587	Sutherland et al. 2011

Pyropia cinnamomea		EU521637	AH008010	Sutherland et al. 2011
Pyropia columbina		GU046423	GU046398	Sutherland et al. 2011
Pyropia conwayae	HQ699188	EU223045	GU319838	Sutherland et al. 2011
Pyropia dentata		HQ687520	HQ687588	Sutherland et al. 2011
Pyropia denticulata		HQ687521	HQ687570	Sutherland et al. 2011
Pyropia fallax	JN028601	GU319865	GU319840	Sutherland et al. 2011
Pyropia fucicola	JN028613	EU223088	GU319841	Sutherland et al. 2011
Pyropia gardneri AK	JN028643	EU223096	GU319842	Sutherland et al. 2011
Pyropia haitanensis	KP266600	AB118585	AB013181	Sutherland et al. 2011
Porphyra hiberna		GU319866	GU319843	Sutherland et al. 2011
Pyropia hollenbergii		HQ687523	HQ687589	Sutherland et al. 2011
Pyropia ishigecola		HQ687524	HQ687571	Sutherland et al. 2011
Pyropia kanakaensis		EU223099	GU319844	Sutherland et al. 2011
Pyropia katadae JP		HQ687525	HQ687572	Sutherland et al. 2011
Pyropia katadae KOR		HQ728199	HQ728191	Sutherland et al. 2011
Pyropia kinositae		EU52164	EU521640	Sutherland et al. 2011
Pyropia koreana		HQ728198	HQ728190	Sutherland et al. 2011
Pyropia kuniedae		HQ728200	HQ728192	Sutherland et

				al. 2011
Pyropia kurogii AK	JN028655	EU223105	GU319845	Sutherland et al. 2011
Pyropia kurogii JP		HQ687526	HQ687573	Sutherland et al. 2011
Pyropia lacerata		HQ687527	HQ687574	Sutherland et al. 2011
Pyropia leucosticta (A)	AM943398	HQ687528	HQ687593	Sutherland et al. 2011
Pyropia moriensis		EU521645	EU521644	Sutherland et al. 2011
Pyropia nereocystis	JN028685	EU223117	GU319849	Sutherland et al. 2011
Pyropia onoi		HQ687529	HQ687575	Sutherland et al. 2011
Pyropia pendula		HQ687530	DQ084430	Sutherland et al. 2011
Pyropia perforata	JN028708	EU223127	AY909592	Sutherland et al. 2011
Pyropia pseudolanceolata	HQ699176	EU223145	GU319857	Sutherland et al. 2011
Pyropia pseudolinearis JP		HQ687531	HQ687590	Sutherland et al. 2011
Pyropia pseudolinearis KOR		HQ728196	HQ728188	Sutherland et al. 2011
Pyropia pulchella		HQ687532	HQ687591	Sutherland et al. 2011
Pyropia rakiura		EU521646	AF136425	Sutherland et al. 2011
Pyropia saldanhae	KY814931	GU165838	AY292630	Sutherland et al. 2011
Pyropia seriata		HQ687533	HQ687576	Sutherland et al. 2011
Pyropia pulchra (*smithii*)	JN028779	EU223224	GU319861	Sutherland et al. 2011;

				Lindstrom and Hughey, 2016
Pyropia protolanceolata (480)		GU319867	GU319846	Sutherland et al. 2011; Lindstrom et al. 2015a
Pyropia columbiensis (485)		GU319868	GU319847	Sutherland et al. 2011; Lindstrom et al. 2015a
Pyropia unabbottiae (523)		GU319869	GU319853	Sutherland et al. 2011; Lindstrom et al. 2015b
Pyropia sp. 551		GU319870	GU319854	Sutherland et al. 2011
Pyropia sp. AKL		GU046403	GU046402	Sutherland et al. 2011
Pyropia sp. Antar68		HQ605698	HQ605699	Sutherland et al. 2011
Pyropia sp. DRB		HQ687534	AY909599	Sutherland et al. 2011
Pyropia sp. FIA	KP781603	GU165842	AY292637	Sutherland et al. 2011
Pyropia sp. FIC		GU046422	AY292638	Sutherland et al. 2011
Pyropia sp. FID		GU046406	GU046396	Sutherland et al. 2011
Pyropia sp. FIE		GU046408	AH015106	Sutherland et al. 2011
Pyropia bajacaliforniensis (MIG=FAL)		HQ687536	DQ084426, DQ084427	Sutherland et al. 2011
Pyropia francisii (PTK)		HQ687537	HQ687592	Sutherland et al. 2011
Pyropia pilcata (ROS054)		GU046410	AF136426	Sutherland et

				al. 2011
Pyropia sp. ROS125		HQ687538	AY184352 & AY184353	Sutherland et al. 2011
Pyropia sp. SMR		HQ687539	AY909587	Sutherland et al. 2011
Pyropia sp. SSR053		GU046411	AF136427	Sutherland et al. 2011
Pyropia sp. SSR091		GU046421	AF136428	Sutherland et al. 2011
Pyropia sp. STI		HQ687540	AY909584	Sutherland et al. 2011
Pyropia sp. TCH		GU046418	AY909583	Sutherland et al. 2011
Pyropia sp. WRO		HQ687541	AY909586	Sutherland et al. 2011
Pyropia sp. RSAk (ZLI)	KY814951	GU165839	AY292635	Sutherland et al. 2011
Pyropia spiralis	JN222758	HQ605696	AY766360	Sutherland et al. 2011
Pyropia suborbiculata		HQ728201	HQ728193	Sutherland et al. 2011
Pyropia tanegashimensis	JN222752	HQ687542	HQ727887	Sutherland et al. 2011
Pyropia tenera	AB477377	HQ687543	HQ687577	Sutherland et al. 2011
Pyropia tenuipedalis		EU521649	EU521648	Sutherland et al. 2011
Pyropia torta	JN028802	EU223236	GU319863	Sutherland et al. 2011
Pyropia vietnamensis	JN222751	HQ687544	HQ687578	Sutherland et al. 2011
*Pyropia viridident*ata		EU521650	AF136421	Sutherland et al. 2011
Pyropia yezoensis	JN028803	HQ728197	HQ728189	Sutherland et al. 2011

Wildemania amplissima	HM917057	HQ687560	HQ687585	Sutherland et al. 2011
Wildemania norrissii		EU223212	GU319850	Sutherland et al. 2011
Wildemania occidentalis	HQ699214	EU223118	GU319851	Sutherland et al. 2011
Wildemania schizophylla	HQ699237	GU319871	GU319860	Sutherland et al. 2011
Wildemania sp. Antar23		HQ605700	HQ605701	Sutherland et al. 2011
Wildemania sp. FII	KP781664	GU165883	GU165844	Sutherland et al. 2011
Wildemania sp. HM080		HQ728202	HQ728194	Sutherland et al. 2011
Wildemania variegata AK		EU223237	GU319864	Sutherland et al. 2011
Wildemania variegata JP		GU046430	GU046401	Sutherland et al. 2011
Described or identified as unique				
'*Bangia*' sp. 1		AF043368.1		Müller et al. 1998
'*Bangia*' sp. 363		EU223010.1		Lindstrom et al. 2008
'*Bangia*' sp. sddy		FJ769174.1		Unpublished[1]
Boreophyllum aleuticum		KT936157.1		Lindstrom et al. 2015b
Boreophyllum ambiguum		KT936164.1		Lindstrom et al. 2015b
Fuscifolium sp. CHA	KP781631			Guillemin et al. 2016
Miuraea sp. CDN-2004		AY795901.1		Unpublished[1]
Porphyra corallicola	JN028496	JN028943		Kucera & Saunders, 2012
Porphyra sp. FO		JN787112		Mols-

				Morstensen et al. 2012
Porphyra sp. CHB	KP781663	KP781808.1		Guillemin et al. 2016
Porphyra sp. CHC	KP781644	KP781811.1		Guillemin et al. 2016
Porphyra sp. CHD	KP781673			Guillemin et al. 2016
Porphyra sp. CHE		KP781707.1		Guillemin et al. 2016
Porphyra sp. FIH	JN028551	KP781853.1		Guillemin et al. 2016
Porphyra RSAd	KX852797	KX852966	XX	This study
Porphyra RSAac	KX852801	KX852968	XX	This study
Porphyra RSAj	KX852809	KX852971	XX	This study
Porphyra RSAg	KX852849	KX852982	XX	This study
Porphyra RSAf	KX852785	KX853000	XX	This study
Porphyra RSAc	KX852935	KX853009	XX	This study
Porphyra RSAh	KX852955	KX853015	XX	This study
Pyropia sp. #5		EU223190		Lindstrom et al. 2008
Pyropia sp. P10		AB366146.1		Niwa et al. 2009
Pyropia montereyensis (1Cal)	JN028782	JN028999.1	KP903907.1	Lindstrom et al. 2015a (Kucera & Saunders, 2012)
Pyropia peggicovensis (Bangiales sp. HK-2011d)		JN028990.1		Kucera & Saunders, 2012
Pyropia sp. 2Cal	JN028791	JN029002.1		Kucera & Saunders, 2012
Pyropia sp. 6POR	JN028792	JN029003.1		Kucera &

				Saunders, 2012
Pyropia raulaguilarii		JQ684700.1	JQ684704	Mateo-cid et al. 2012
Pyropia njordii	JN028690	JN847259.1		Mols-Morstensen et al. 2012
Pyropia '*leucosticta*' *B*	DQ442890			Mols-Morstensen et al. 2012
Pyropia taeniata		KT936189.1		Lindstrom et al. 2015b
Pyropia orbicularis	KP781667	KF479481.1		Ramirez et al. 2014
Pyropia parva		KJ182946.1	KJ395115.1	Sanchez et al. 2014
Pyropia sp. GCII		JQ900565.1	JQ900551	López-Vivas et al. 2015
Pyropia sp. GCIII		JQ900554.1	JX024906	López-Vivas et al. 2015
Pyropia nitida	KR139854.1	KP876025.1		Harden et al. 2016
Pyropia sp. CHG		KP781837.1		Guillemin et al. 2016
Pyropia sp. CHH	KP781676	KP781845.1		Guillemin et al. 2016
Pyropia sp. CHI	KP781620	KP781715.1		Guillemin et al. 2016
Pyropia sp. CHJ	KP781662	KP781815.1		Guillemin et al. 2016
Pyropia sp. CHK	KP781619	KP781747.1		Guillemin et al. 2016
Neothemis ballesterosii		KJ182954.1	KJ395110.1	Sanchez et al. 2014
Neothemis iberica		KJ182960.1	KJ395111.1	Sanchez et al.

Wildemania sp. 5POR	JN028552	JN028952.1	2014 Kucera & Saunders, 2012
Pyropia sp. DN002		AB287968.1	Unpublished[1]
Pyropia sp. #3		EU223139.1	Unpublished[1]
Pyropia sp. DN001		AB287962.1	Unpublished[1]
Pyropia sp. P7		AB366143.1	Unpublished[1]
Pyropia sp. ARC	KP998749		
Wildemania sp. HK2011	JN028920		
Unpublished names			
Pyropia sp. *kanyakumariensis*	KP998745		Unpublished
Pyropia sp. *spatulata*		DQ813635.1	Unpublished[1]
Pyropia sp. *novae-angliae*		DQ813608.1	Unpublished[1]
Porphyra sp. *oligospermatangia*		GQ427213	Unpublished[1]
Published names but included in Sutherland			
Porphyra mufordii	JN028497	KP781809.1	Guillemin et al. 2016
Pyropia thulaea		JN847268.1	Unpublished[1]
Pyropia elongata		JN847254	Unpublished[1]
Wildemania abyssicola		JN847270.1	Unpublished
Wildemania miniata	HM915243	JN847276.1	Unpublished

Unpublished[1] : cited in Kucera and Saunders (2005)

Table S6. Neutrality tests for each zone based on *cox*1 sequence data.

Species	**N**	**Hd**	**Nd**	**Tajima's D**	**Fu's Li D**	**Fu's Li F**	**Fu's Fs**	**R^2**
RSAa								
Zone 1	11	<1	<1	<1	<1	<1	<1	
Zone 2	12	56	0.0237	-0.38	0.08	-0.03	-0.89	0.1502
Zone 3	7	71	0.01606 (0.127)	0.21	-0.06	0	-0.24	0.2259
False Bay								0.4714
RSAb								
Zone 3	9	78 (0.11)	0.003 (<0.01)	-0.71	-1.02	-1.05	0.55	0.2368
False Bay	14	76 (0.11)	0.005 (<0.01)	-0.57	-0.69	-0.76	-0.68	0.1160
East of Cape Hangklip	24	60 (0.11)	0.003 (<0.01)	-0.92	-0.61	-0.813	-0.93	0.0886
Zone 4								0.2000
RSAe								
Zone 1	9	56 (0.12)	<0.001 (<0.01)	-0.58	-0.22	-0.34	-0.53	0.1848
Zone 3	14	14 (0.12)	<0.001 (<0.01)	-1.15	-1.40	-1.51	-0.59	0.2575
P. agulhensis								
South coast	25	16 (<0.01)	<0.001 (<0.01)	-1.51	-2.18	-2.30	-2.12	0.1356
South-east coast	23	53 (0. 01)	0.001 (<0.01)	**-2.23****	**-3.18****	**-3.37****	-4.463	0.0704

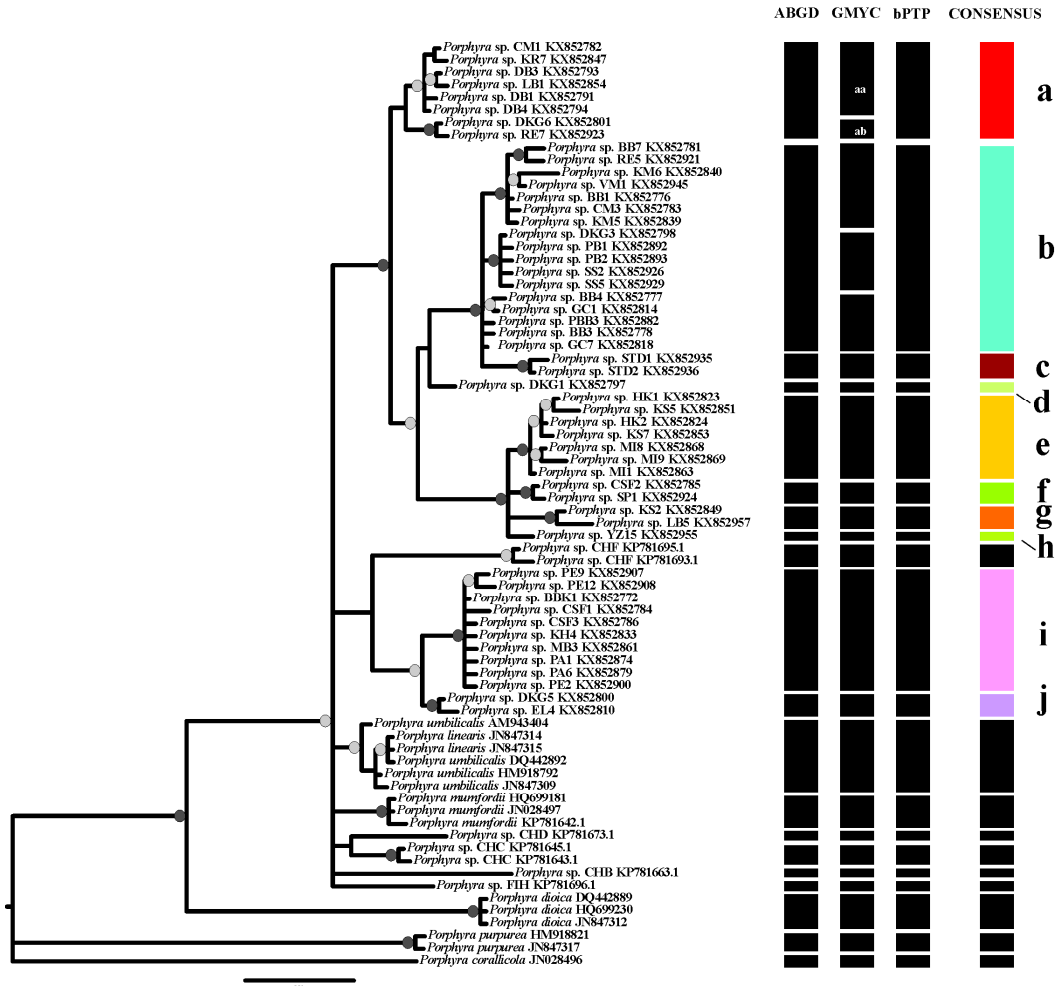

Figure S1: Phylogenetic gene tree based on the *cox*1 gene for the genus *Porphyra* including South African sequences generated in this study. Bayesian topology is presented and was congruent with RAxML. Dark circles represent high clade support for both methods (BI and ML) with values of posterior probability (> 0.80) and ML bootstrap values (> 80). Light circles represent nodes that were well supported for only a single method. Three species delimitation methods are indicated on the figure; individual species are represented as bars for each of the methods. A consensus column denotes species groupings based on a 50% majority rule. For South African taxa, each unique species hypotheses are given a unique colour and label (a-j).

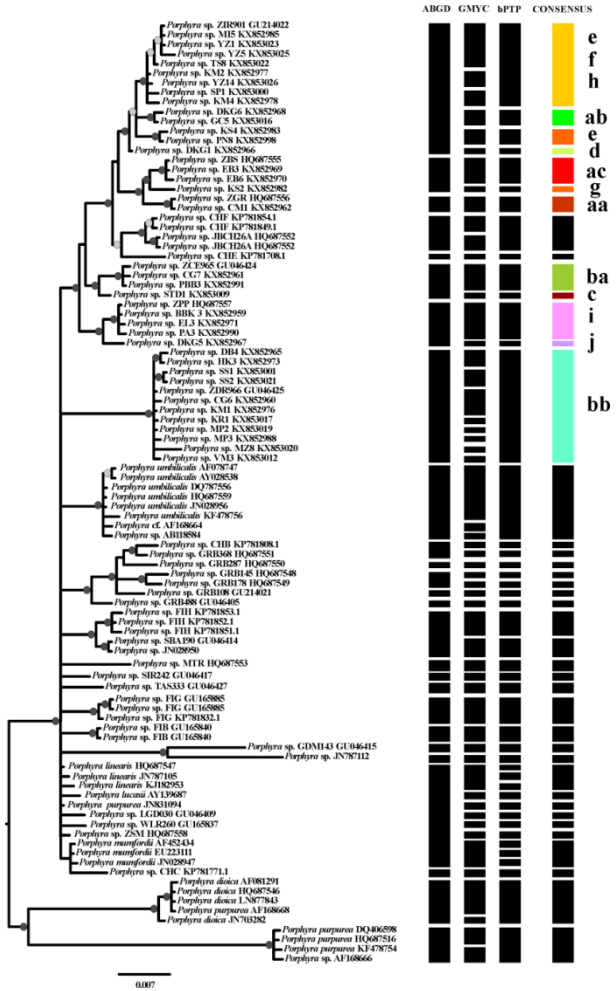

Figure S2: Phylogenetic gene tree based on the *rbc*L gene for the genus *Porphyra*. Bayesian topology is presented and was congruent with RAxML. Dark circles represent high clade support for both methods (BI and ML) with values of posterior probability (> 0.80) and ML bootstrap values (> 80). Light circles represent nodes that were well supported for only a single method. Three species delimitation methods are indicated on the figure; individual species are represented as bars for each of the methods. A consensus column denotes species groupings based on a 50% majority rule. For South African taxa, each unique species hypotheses are coloured and labelled according to the *cox*1 consensus (a-j). Where a single species hypothesis was paraphyletic e.g. “a”, it was labelled as aa, ab and ac.

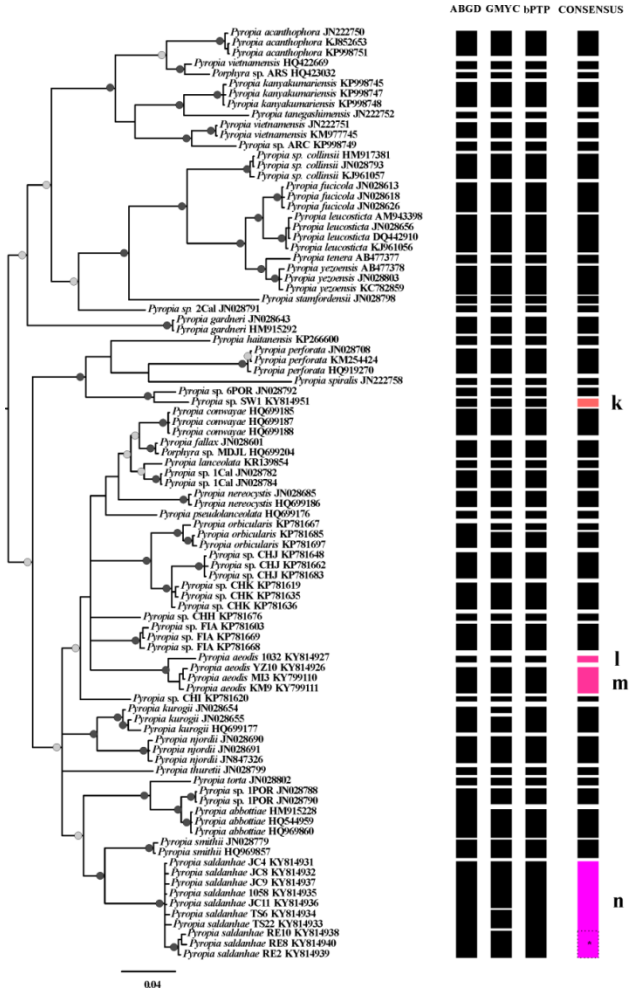

Figure S3: Phylogenetic gene tree based on the *cox*1 gene for the genus *Pyropia*. Bayesian topology is presented and was congruent with RAxML. Dark circles represent high clade support for both methods (BI and ML) with values of posterior probability (> 0.80) and ML bootstrap values (> 80). Light circles represent nodes that were well supported for only a single method. Three species delimitation methods are indicated on the figure; individual species are represented as bars for each of the methods. A consensus column denotes species groupings based on a 50% majority rule. For South African taxa, species hypotheses are given a unique colour and label (k-n*). "*" denotes a divergent lineage.

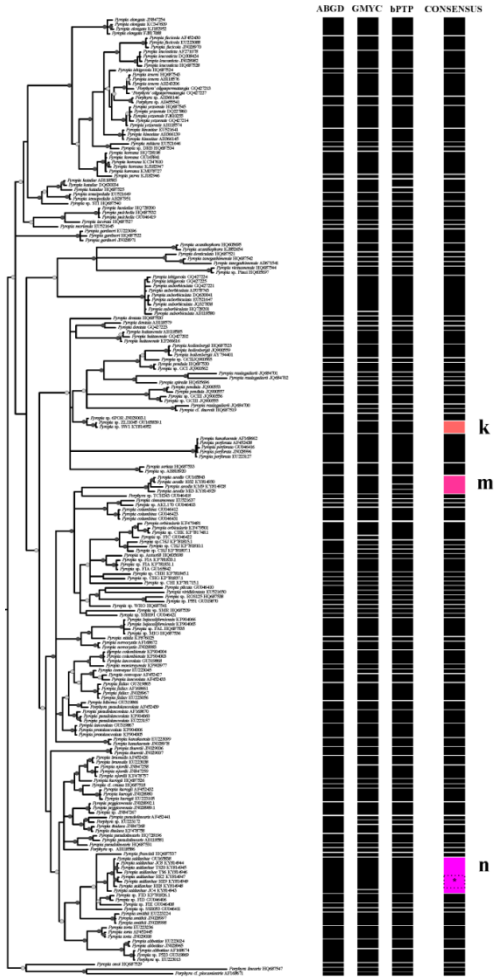

Figure S4: Phylogenetic gene tree based on the *rbc*L gene for the genus *Pyropia*. Bayesian topology is presented and was congruent with RAxML. Dark circles represent high clade support for both methods (BI and ML) with values of posterior probability (> 0.80) and ML bootstrap values (> 80). Light circles represent nodes that were well supported for only a single method. Three species delimitation methods are indicated on the figure; individual species are represented as bars for each of the methods. A consensus column denotes species groupings based on a 50% majority rule. For South African taxa, species hypotheses are coloured and labelled according to the *cox*1 consensus (k, m, n, & n*). "*" denotes a divergent lineage.

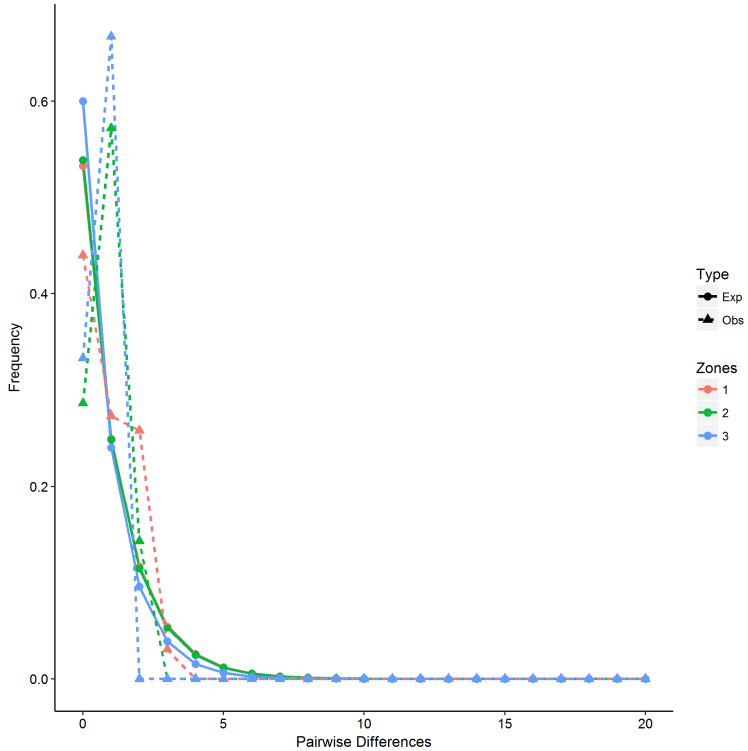

Figure S5. Mismatch distribution plots for *Porphyra* RSAa from various locations.

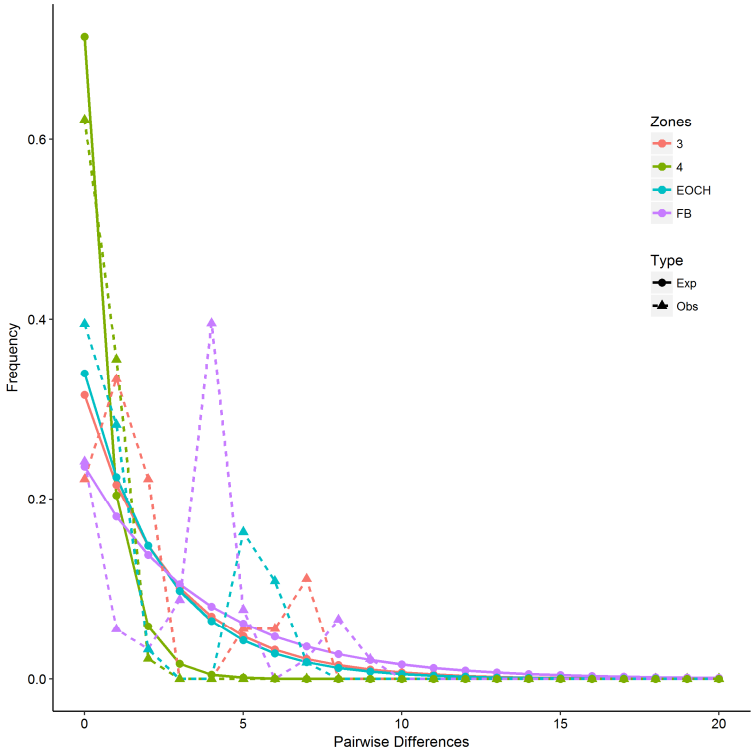

Figure S6. Mismatch distribution plots for *Porphyra*RSAb various locations.

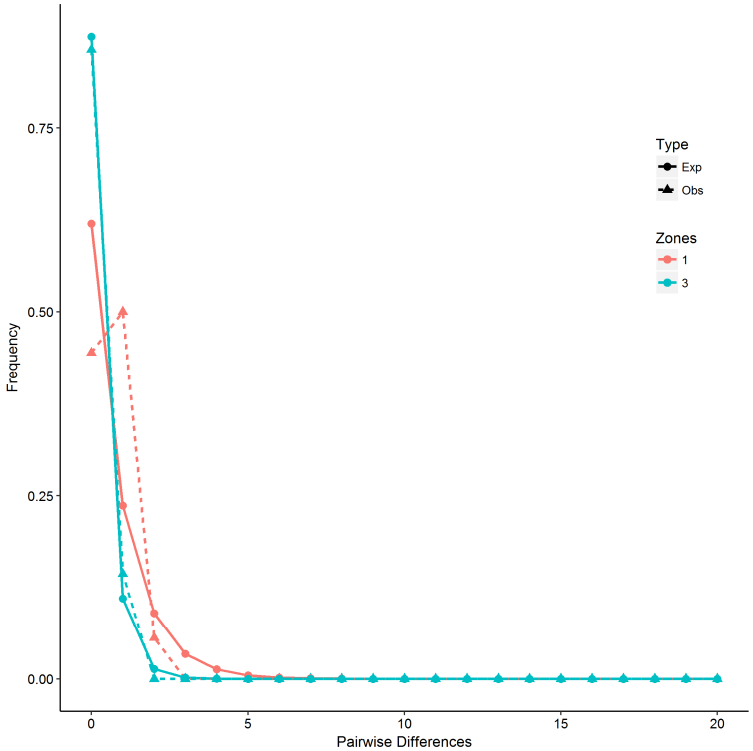

Figure S7. Mismatch distribution plots for *Porphyra* RSAe from various locations.

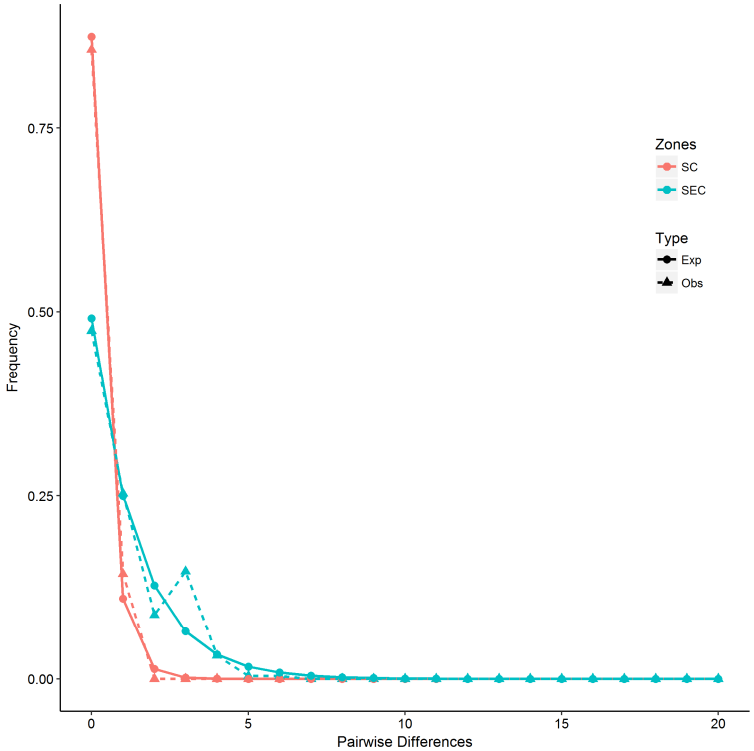

Figure S8. Mismatch distribution plots for *P. agulhensis* from various locations.

Note S1: Clusters identified using *a prior* (ANOVA and DA) and no *prior* (nMDS and cluster diagrams) for morphological and ecological traits (length, weight, position of holdfast, shape, texture, colour, shore position and distribution) for the full dataset were not consistent with molecular species groups (not shown).

Note S2: Analyses for the anatomical characters were obtained for a subset of the data. Reproductive cells were not well represented for each species and generally correlated with vegetative characters; therefore these data were not included. For the vegetative cells, cell width correlated with cell height and these were also removed from the analyses.